The physiological and genetic basis of yield improvement in an elite barley line adapted to Australian conditions

A thesis submitted to The University of Adelaide in fulfilment of the requirements for the degree of Doctor of Philosophy

Kenton Dean Porker

School of Agriculture, Food and Wine

The University of Adelaide

February 2018

Table of Contents

Table of Contents 2

Abstract 4

Declaration 6

Acknowledgements: 7

List of publications and presentations 8

Structure of the thesis 10

CHAPTER 1: General Introduction 11

Project aims and experimental approach: 14

CHAPTER 2: Literature Review 15

CHAPTER 3: Using a novel PLS approach for envirotyping of barley phenology and adaptation 42

Using a novel PLS approach for envirotyping of barley phenology and adaptation 45

Abstract: 45

Introduction: 46

Materials and Methods 50

Results: 54

Discussion: 65

Conclusion: 70

References: 71

Supplementary Tables & Figures 78

CHAPTER 4: Developmental variation driving yield and adaptation of barley to Mediterranean environment 83

Abstract 87

Introduction 88

Materials/Methods 91
Results: 96
Discussion: 109
References: 116
Supplementary tables and figures: 120
CHAPTER 5: Genetic analysis of yield and adaptation in the narrow bi-parental barley population Commander x Compass. 128
Abstract: 131
Introduction: 132
Materials and Methods: 135
Results: 140
Discussion: 158
References: 165
Supplementary Tables and Figures 169
CHAPTER 6: General Discussion, main findings and conclusions 177
General Discussion 178
Other considerations 183
Recommendations 184
Concluding remarks 185
Appendix 1: Is Vrn-H1 a missed opportunity for southern Australian barley growers? 187

Abstract

Genetic improvement in yield potential is a primary objective of barley breeding programs. The variety 'Compass' represents a step change in yield potential, showing a consistent yield improvement over the malting benchmark variety Commander across different environments. The objective of this study was to identify the physiological and genetic bases of improved yield and adaptation of Compass. Crop development is considered the most important factor affecting grain yield and adaptation in dry land Mediterranean cropping systems. It was hypothesised that the improved yield of Compass was due to differences in the pattern of crop development despite Compass being genetically similar to and derived from Commander.

Using partial least regression (PLS) the environmental modulation of flowering time in three elite barley cultivars, Compass, Commander, and Fathom, was described across 35 environments at a range of sowing times commonly used by growers in southern Australia encompassing a wide range of temperature and photoperiod regimes. This analysis gave insight into the subtle responses to changes in temperatures that are not adequately accounted for in current crop simulation models. The results suggested that under short photoperiods, varietal responses to temperature might be equally as important as photoperiod in determining time to flower. Based on field trial observations it was concluded that through selective breeding, breeders have shortened the time to anthesis and the duration of the pre-anthesis phases without compromising yield potential in the variety Compass. Compass had a faster development rate and its improved yield was associated with modest improvements in grain number with heavier grain weight. The development of the Commander x Compass bi-parental mapping population allowed genetic analysis of the quantitative trait loci (QTL) controlling developmental and yield traits. The QTL for developmental traits were predominantly located near the candidate photoperiod response gene (*Ppd-H1*) on chromosome 2H. The faster development of Compass at May-June sowing dates was due to reduced responsiveness to photoperiod. Although there were QTLs of smaller effect that were independent of photoperiod. Current breeding programs have historically focussed on developing photoperiod-responsive varieties, but the reduced photoperiod sensitivity of Compass suggests an alternative means of improving yield potential. Two QTL identified on 4H and 6H were associated with larger grain weight and it was possible to improve grain weight in cultivars of similar heading time and in contrasting photoperiod sensitivity groups.

These results present new findings relevant to improving the yield of barley in southern Australia through understanding of the crop's photoperiod and temperature responses. The results of this study highlight that breeders should consider selecting for a diverse range of phenology genes and it is possible to improve yield and kernel weight within a narrow flowering range. The results from this study reflect the most up-to-date information on the importance of phenology to yield adaptation in southern Australian environments. This information will assist in developing more accurate flowering models and facilitate further fine-tuning of crop development and yield improvement under the short photoperiods associated with autumn planting dates in southern Australian environments.

Declaration

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

I acknowledge that copyright of published works contained (see named in "list of publications and presentations) within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Kenton Dean Porker Date:

Acknowledgements:

I would like to acknowledge the support I received from my supervisors; Jason Eglinton, for believing an agronomist could learn genetics; Stewart Coventry, for robust discussions about all things barley; Neil Fettell, who ran interstate field trials and always made time for a late night phone call; and Glenn McDonald, who came on board as a supervisor late in the piece and was able to offer excellent objective advice throughout the write up phase.

Special thanks to the members of the University of Adelaide Barley Research Lab and Barley Breeding Program for all their help in running experiments. I was fortunate to be surrounded by supportive and professional colleagues. I would like to thank Daniel Cozzolino for teaching me about multivariate analysis, Michael Laws for collecting plants from Loxton trial sites, Tim March for conducting genotyping and his sound advice, and Michael Zerner for all the office support. Julian Taylor from SAGI provided invaluable assistance in trial design.

Further afield, thanks to Ben Travaskis and Kerrie Ramm from CSIRO in Canberra for expanding my horizons into genetic research. Thanks to Dominique Vequaud from Secobra Plant Breeders for facilitating collaboration and his hospitality during my visit to Europe.

Without funding and scholarship support, it would not have been possible to undertake a PhD. I gratefully acknowledge an Australian Postgraduate Award (APA) from the University of Adelaide, the Grains Research and Development Corporation (GRDC) for the Grains Industry Research Scholarship, and the Playford Trust administered the Thyne Reid Foundation PhD scholarship. Thanks to the Farrer Memorial Trust for a travelling scholarship that enabled me to attend the International Barley Genetics Symposium in Minneapolis in 2016 and to Chengdao Li (Murdoch University) for operating funds from the GRDC Manipulating barley phenology to maximise yield potential project.

The South Australian Research and Development Institute (SARDI) deserves special mention for taking the chance on employing me as a Research Scientist prior to completion of my PhD. I have appreciated the understanding and support of SARDI Sustainable Systems and all colleagues in the New Variety Agronomy team when I have at times juggled work and study.

I would like to thank my network of family and friends who have supported me along the way even though I have been very anti-social during the final year. Thanks to my parents who believed in my education. Finally, I would like to thank my wife Kimberley for all her care, encouragement, understanding and support throughout my candidature.

List of publications and presentations

Research and scientific

Porker, K., Eglinton, J., Coventry, S. & Fettell, N. (2016).Chapter 10 - Improvement of Yield and Adaptation by Manipulating Phenology Genes. In Exploration, Identification and Utilization of Barley Germplasm, 241-264: Academic Press.

Porker, K., Eglinton, J., Coventry, S. & Fettell, N. (2015). Opportunities to Improve Barley Yield by Optimising Development Pattern., Proceedings of the 2015 17th Australian Barley Technical Symposium., Manly, NSW Australia (paper and oral presentation)

Porker K, Coventry S, Fettell NA, Eglinton JK. 2016. Raising yield potential in barley- Proceedings of the 12th International Barley Genetics Symposium (IBGS) 26-30 June 2016. Minnesota, USA (Oral and Poster Presentation)

Porker K, Coventry S, Trevaskis B, Fettell NA. 2017. Is Vrn-H1 a missed opportunity for southern Australian barley growers? Doing More with Less - Proceedings of the 18th Australian Society of Agronomy Conference, 24 – 28 September 2017. Ballarat, VIC, Australia: Australian Society of Agronomy. (Paper and oral presentation)

Porker K, Coventry S, Trevaskis B, Fettell NA. 2017. Winter barley - an early sowing opportunity for southern Australian barley growers - Proceedings of the 18th Australian Barley Technical Symposium (ABTS), September 2017. Hobart, TAS Australia (paper and oral presentation).

Porker K. 2016. A new development pattern for Australian barley, CSIRO Developing Crops of the Future Workshop, Kiama NSW (Invited Speaker)

Industry meetings and reports

Angessa, T., Porker K., Gaofeng Z., Moeller C., Li C. 2016. Adaptation of barley varieties to Australian barley growing environments: An Industry report prepared for the Grains Research and Development Corporation (GRDC), Murdoch University, Western Australia, Australia.

Porker K, Fettell N., Coventry S., Chong P., McDonald G., Eglinton J., (2017) Drivers of Barley Yield in Southern Australia, GRDC Update Paper Adelaide Advisor Updates 2017. URL:https://grdc.com.au/resources-and-publications/grdc-update-papers/tab-content/grdc-update-papers/2017/02/drivers-of-barley-yield-in-southern-australia (paper and oral presentation)

Porker K., August 2015 "Crop development and adaptation", Coopers Brewery SA, South Australian Barley Advisory Committee Spring meeting (oral presentation)

Porker K., July 2015, "Flowering time behaviour in barley varieties", Roseworthy, SA Crop Science Society Meeting. (Oral presentation)

Structure of the thesis

This thesis is presented in publication format and includes papers that have been prepared for submission to a journal. All papers are based on extensive field experimentation.

Chapter 1 introduces the thesis and gives an overview and general discussion on the rationale to the project and the importance of crop phenology in the context of barley in Australia. The chapter concludes with the aims and objectives of my thesis.

Chapter 2 is the literature review titled "Improvement of Yield and Adaptation by Manipulating Phenology Genes" and was published in Exploration, Identification and Utilization of Barley Germplasm. This was written as part of my initial research proposal in 2014 and analyses the current literature up to the start of the current study. As a result, publications that are more recent are not included in the Literature Review but are discussed where relevant in the introduction and discussion sections of the following chapters.

Chapter 3 Presents a novel modelling approach for envirotyping of barley phenology and adaptation and describes the environmental modulation of flowering time in three elite barley cultivars, Compass, Commander, and Fathom, across 35 environments encompassing a wide range of temperature and photoperiod regimes in southern Australia.

Chapter 4 aims to describe the link between crop development and yield in Compass and elite barley lines from extensive field experimentation and phenotypic analysis.

Chapter 5 is based on the three year development of an elite Commander x Compass bi-parental population and aims to identify major quantitative trait loci (QTL) for the developmental and yield trait differences between Compass and Commander traits that were previously discussed in detail in chapters 3 and 4.

Chapter 6 is the General Discussion, which synthesises the key findings of the thesis articles and concludes with recommendations for future research on this topic

The appendix comprises a collection of conferences papers and posters presented throughout the course of the PhD.

CHAPTER 1: General Introduction

Background and project significance

This project aims to describe the physiological and genetic bases of yield improvement in an elite barley line "Compass" adapted to Australian conditions. Barley *(Hordeum vulgare* L.*)* is the second most important winter cereal crop in Australia with current barley production between 2015-2018 covering on average 4.07 M Ha and producing 10.45 Mt with an average yield of 2.6t/ha (ABARE, 2018). Plant breeders have made significant yield improvement with the successive release of cereal cultivars better adapted to Australian environmental conditions. It is widely recognised that improved adaptation is achieved by matching crop phenology with availability of resources to maximise crop growth and to avoid abiotic stresses associated with particular weather conditions at critical stages of development. In many Mediterranean environments, selection for yield and appropriate flowering date to avoid frost, heat and water stress has resulted in yield improvement in modern cultivars (Peltonen-Sainio *et al.*, 2007, Richards, 1991). In environments such as southern Australia, flowering must occur within a relatively narrow flowering window defined by the frequency of frost and heat/drought stress. Consequently, there is little scope for breeders to make large adjustments to the time of flowering but there are greater opportunities to alter times of sowing and to alter pre-anthesis development. Therefore, there is increasing focus on the development physiology prior to anthesis and especially on stages of development that are important for yield formation. One approach for future yield improvement is to fine-tune rates of development within elite material of similar flowering time. This could be achieved through selective breeding for variation in pre-anthesis phase duration; however, we currently do not have the knowledge on the genetic and physiological level to improve breeding efficiency in selection for such physiological traits. The most favourable combinations of developmental genes conferring adaptation are also not well defined, particularly with respect to their role in yield component generation and control of the duration of pre anthesis phases. Previous studies that investigated the pre-anthesis phases of development in Australian cultivars were conducted using older cultivars and did not link field performance with genotypic data.

At the beginning of the project, the phenological pattern that confers superior adaptation of high yielding barley varieties to Australian environments was not well established. The variety Compass was developed by the University of Adelaide breeding program and released for commercial production in 2015. Compass was developed from the progeny of a backcross between the current leading benchmark malting barley variety in Australia, Commander, and

a European introduction, County. In widespread evaluation through the Australian National Variety trials (NVT) until 2014, Compass was on average 10% higher yielding than Commander and was ranked among the highest yielding varieties in every state of the NVT program (Figure 1). Despite being from a relatively narrow genetic base, this is a significant advancement in yield performance and adaptation and is a step change by commercial breeding standards. Compass therefore represented the best 'model' variety for dissection of the genetic and physiological bases of improved yield for adaptation to southern Australian. Understanding the reasons for the yield improvement of Compass may allow the identification of new pathways for future yield improvement.

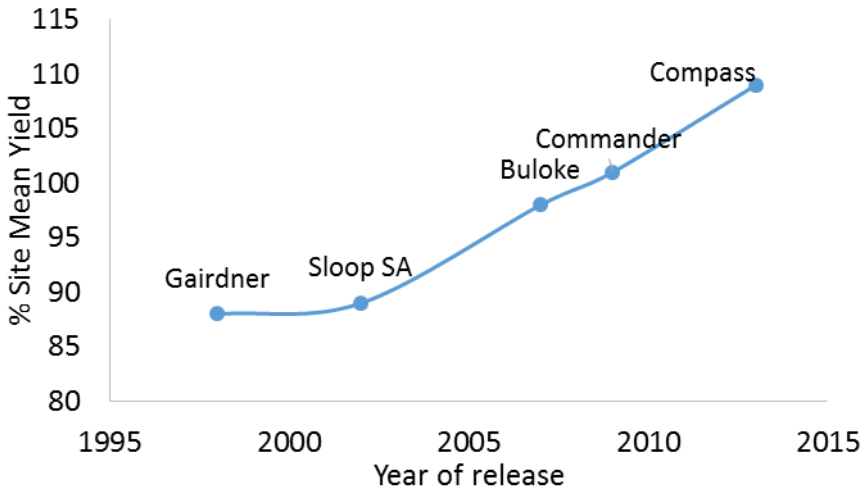


Figure 1 Grain yield of selected barley varieties compared to year of release and relative to the site mean yield from the 2013 SA National Variety Trials multi environment analysis (NVT Online)

Given the importance of crop development for adaptation, the main research focus was based on understanding the environmental and genetic modulation of crop development in Compass. In breeding trials sown from late autumn to winter, Compass flowered at a similar time or a few days earlier than Commander did. However, in summer nurseries greater differences were noted between Commander and Compass, including sister lines derived from the same cross; Compass and its siblings were found to flower significantly later than Commander. Plant breeders have normally favoured lines that flowered early in summer nurseries, as these were considered best suited to Australian environments. The phenology of Compass represented a departure from the normal pattern of development. The different pattern of development in Compass and its high yield and wide adaptation in NVT trials led to the hypothesis that its improved yield was due to subtle differences in crop development pattern leading to improved synchronisation of critical growth stages with the environment. However, there were no detailed studies of development and yield formation of Compass and other elite germplasm in

southern Australia and it was this need to understand why Compass shows a consistently higher yield over a range of environments that prompted the study described in this thesis.

The literature review will first describe the major developmental stages of barley and then seek to discuss the relationship between crop phenology and yield development. The second part of this review will focus on the knowledge gaps of the crop development patterns of cultivars adapted to Australian conditions and identify the potential for improvements in yield by manipulating the phenological profile.

Project aims and experimental approach:

This project aims to understand the genetic control and environmental modulation of crop phenology contributing to a substantial increase in yield performance of a new variety Compass. Identifying both the physiological and genetic bases for increased yield and adaptation will improve agronomic management, identify new opportunities for increasing yield and facilitate a selection mechanism better suited to breeding programs than physiological screening.

The experimental approach to this project utilises a Genetic x Environment x Management (GxExM) framework encompassing multidisciplinary tools such as crop modelling, a multi-environment trial network, and detailed physiological and genetic analysis. The details are outlined in Chapters 3 – 5. Agronomic field trials across multiple sowing times were conducted in 2014 and 2015 to investigate phenology and yield responses reported in Chapter 3 and 4. Chapter 5. describes studies using a bi-parental Compass x Commander population that was developed and planted in field trials at three sowing times at Roseworthy, South Australia to determine the quantitative trait loci controlling development and their association with agronomic traits related to grain yield.

CHAPTER 2: Literature Review

Statement of Authorship

Title of Paper	Improvement of Yield and Adaptation by Manipulating Phenology Genes
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Porker, K., Eglinton, J., Coventry, S. & Fettell, N. (2016).Chapter 10 - Improvement of Yield and Adaptation by Manipulating Phenology Genes. In Exploration, Identification and Utilization of Barley Germplasm, 241-264: Academic Press.

Principal Author

Name of Principal Author (Candidate)	Kenton Porker		
Contribution to the Paper	Conducted the literature research, wrote the review, and acted as corresponding author		
Overall percentage (%)	85%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	26 February 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Neil Fettell		
Contribution to the Paper	Editing and reviewing manuscript		
Signature		Date	27 February 2018

Name of Co-Author	Jason Eglinton

Contribution to the Paper	Provided historic literature, editing and reviewing manuscript		
Signature		Date	28th February 2018

Name of Co-Author	Stewart Coventry		
Contribution to the Paper	Provided historic literature, editing and reviewing manuscript		
Signature		Date	28TH FEBRUARY 2018

Please cut and paste additional co-author panels here as required.

10

Improvement of Yield and Adaptation by Manipulating Phenology Genes

Kenton Porker*, Jason Eglinton*, Stewart Coventry*, Neil Fettell**

**UNIVERSITY OF ADELAIDE, WAITE CAMPUS, PMB, SA, AUSTRALIA; **CENTRAL WEST FARMING SYSTEMS & UNIVERSITY OF NEW ENGLAND, CONDOBOLIN, NSW, AUSTRALIA*

CHAPTER OUTLINE

1 Introduction 242
2 Barley Development and Physiological Determinants of Yield 242
2.1 Development of the Barley Plant 243
2.2 Barley Development and Yield Component Generation 244
2.3 Defining and Identifying Major Phases of Development 245
3 Control of Barley Development 246
3.1 Environmental and Genetic Controls 246
3.2 Photoperiod 246
3.3 Vernalization 249
3.4 Earliness *per se* 249
3.5 Basic Vegetative Period 250
4 Phenology and Adaptation: Matching Crop Phenology to Growing Conditions in Australia 250
4.1 Growing Regions and Seasonal Conditions 251
4.2 Agronomic and Breeding Significance 252
4.3 Developmental Variation in Australian and Introduced Genotypes 253
4.4 Mapping Studies of Phenologic Variation 253
4.5 Linking Gene Sequence Information With Field Performance 254
5 Manipulating Developmental Phases for Further Yield Improvement 254
5.1 Improving Grain Number 255
5.2 Manipulating the Critical Period 255
5.3 Genetic Control of Preheading Phases 257
5.4 Mapping Studies of Preanthesis Phases 258
6 Conclusions 259
References 259

Exploration, Identification and Utilization of Barley Germplasm

1 Introduction

The pattern of crop development is the single most important factor influencing grain yield and adaptation. Improved adaptation is achieved by matching crop phenology with availability of resources to maximize crop growth and to avoid abiotic stresses associated with weather conditions (Richards, 1991). Crop development is controlled by complex environmental and genetic factors. The major genes that underlie variation in flowering time include variation in response to photoperiod (*Ppd*), vernalisation (*Vrn*), and earliness *per se* (*Eps*). Large variation exists in barley (*Hordeum vulgare* L.) for these genes enabling commercial production during both winter and summer growing conditions in regions away from barley's center of origin in the Mediterranean, which differ not only in temperature but also in altitude and latitudes (Cockram et al., 2007b). As a result, barley has been successfully cultivated in Australia and is the second most important cereal crop, with 2009–2013 barley production averaging 4.09 Mha and 7.91 MT (1.97 t/ha) (ABARE, 2014).

Breeders have made significant yield improvements with the successive release of cereal cultivars better adapted to Australian environmental conditions. Empirical selection for yield and appropriate flowering date to avoid frost, heat, and water stress has resulted in increases in grains per square meter and formed the primary determinant for yield improvement in modern cultivars (Peltonen-Sainio et al., 2007).

Manipulating the preanthesis developmental phase partitioning to optimize grain number prior has been proposed for further yield improvement (Garcia del Moral et al., 2002; Kernich et al., 1997). Recent advances in genomics have enabled access to allelic diversity in developmental genes (Sreenivasulu and Schnurbusch, 2012). Understanding the physiological basis of developmental genes in determining yield will enable targeting of favourable phenology gene combinations for genetic gain.

This review will first describe the major developmental stages of barley and then seek to discuss the relationship between crop phenology and yield development. The second part of this review will focus on the knowledge gaps of the crop development patterns of cultivars adapted to Australian conditions and identify the potential for improvements in yield by manipulating the phenologic profile.

2 Barley Development and Physiological Determinants of Yield

Crop development is a process of phenologic events determined by genetic factors and their interaction with the environment. Although development is a continuous process, the ontogeny (life span) of a crop is frequently divided into discrete periods (Slafer et al., 2009). Development and growth are related processes but can occur independent of one another. Growth can be best defined as an irreversible increase in physical dimensions or dry weight with time (Garcia del Moral et al., 2002). The development of barley can be broadly partitioned into the major phases, vegetative, early reproductive, late reproductive, and grain filling, all of which can be distinguished from each other (Kirby and Appleyard, 1987;

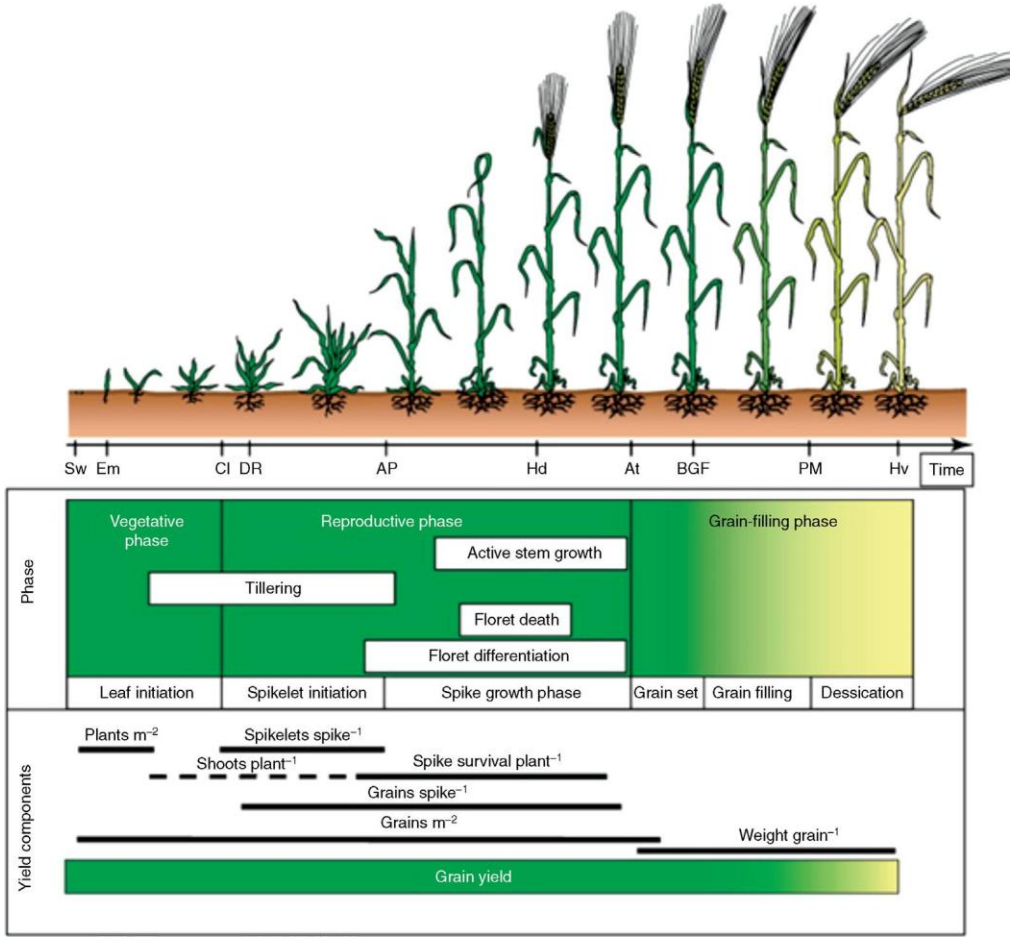


FIGURE 10.1 Schematic diagram of the main stages of barley development throughout the life cycle of the crop on a time scale from sowing to harvest from Sreenivasulu and Schnurbusch (2012). Boxes represent different phases, and developmental processes with relation to the components of grain yield and their interacting overlaps. Abbreviations: AP, awn primordium; At, anthesis; BGF, begin grain filling; CI, collar initiation; DR, double ridge; Em, seedling emergence; Hd, heading time; Hv, harvest; PM, physiologic maturity; Sw, sowing.

Appleyard et al., 1982; Slafer and Rawson, 1994; Sreenivasulu and Schnurbusch, 2012) as shown in the schematic diagram (Figure 10.1).

2.1 Development of the Barley Plant

The *vegetative stage* occurs from emergence up until floral initiation (FI) and is the period in which leaf initiation occurs. The mature barley embryo contains the primordia of the first three to four leaves (Kirby and Appleyard, 1987) and the subsequent initiation of leaf primordial continues until transition to the initiation of spikelet primordial. This transition marks the end of the vegetative phase and the beginning of the *early reproductive*

stage (spikelet or floret initiation), and is commonly characterized by the appearance of double ridges (DR) on the emerging apex. The period from FI until awn primordial development coincides with a rapid increase in rate of spike primordial initiation and concludes after a maximum number of primordia have been initiated and the meristematic growth of the dome has stopped initiating new primordia. The *late reproductive phase* (or spike growth and development phase) occurs from awn primordial initiation up until anthesis. The *grain-filling phase* is the final stage occurring after pollination, wherein the embryo begins to develop and produce a viable seed for a subsequent generation (Kirby and Appleyard, 1987; Garcia del Moral et al., 2002).

2.2 Barley Development and Yield Component Generation

The grain yield of barley is determined by three main components: the number of spikes/ ears per meter square, the number of grains per spike, and the individual grain weight (Garcia del Moral et al., 2002) (Figure 10.1). At each level of integration, components are not independent but are involved in a complex relationship (Sadras and Denison, 2009; Slafer and Rawson, 1994). Phenology plays a major role in the generation of each of these components and therefore yield itself (Hamid and Grafius, 1978).
Grain yield is more closely related to grain number than to grain weight (Peltonen-Sainio et al., 2007). This is maintained even under terminal stress typical of Mediterranean environments (Siddique et al., 1989; Slafer et al., 2014; Garcia del Moral et al., 2002; Sadras and Slafer, 2012). There can be some exceptions, for which grain weight improves yield in barley (Abeledo et al., 2002). The dominance of grain number over grain weight in temperate cereal yield determination results from evolutionary constraints as environmental control of reproductive output relies more on adjustment of seed number, whereas stable seed size is more adaptive (Sadras and Denison, 2009; Sadras and Slafer, 2012).

The reproductive phase is most critical in determination of grain-yield potential as this is when final grain number is set and photo assimilates are converted to yield. At FI, the apical meristem differentiates spikelet structures, which may not progress to form fertile spikelets. The highest frequency of spikelet and floret mortality occurs during the late re-productive phase because this coincides with rapid growth of the stem and spike, causing competition for assimilates (Kirby, 1988; Fischer, 2007; Alqudah and Schnurbusch, 2014). The final number of grains is further determined by floret generation and effectiveness of pollination. Other components, such as number of spikes, are formed constantly and exert compensatory effects on one another (Sadras and Slafer, 2012) (Figure 10.1). The number of spikes per unit area is determined by tiller production and survival occurring from tillering to jointing (Araus et al., 2008).

Grain weight, the third and final yield component, is ultimately determined by the duration and rate of grain filling (Wiegand and Cuellar, 1981), however, it is now recognised that potential grain weight is defined prior to flowering as a function of ovary size, overlapping with grain number determination (Slafer et al., 2014). The plant genotype and

environment- (namely temperature and moisture) control the process of grain filling (Sofield et al., 1977), however, preanthesis development is the focus of this review.

2.3 Defining and Identifying Major Phases of Development

Different stages of plant development can be distinguished using macroscopic scales of external plant appearance (Haunt, 1973; Zadoks et al., 1974) and microscopic scales based on changes in apical morphology (Waddington et al., 1983; Kirby and Appleyard, 1987) (see Figure 10.2). While there is a significant relationship between the external and internal development responses, it is not always constant, clear, reliable, or easily predict-able as the rate of development of the shoot apex, leaves, and tillers respond differently to major environmental cues. External features provide useful indicators about relative growth stages but do not provide an understanding of changes in the shoot apex where development is occurring. There is a good correlation between final leaf number and FI

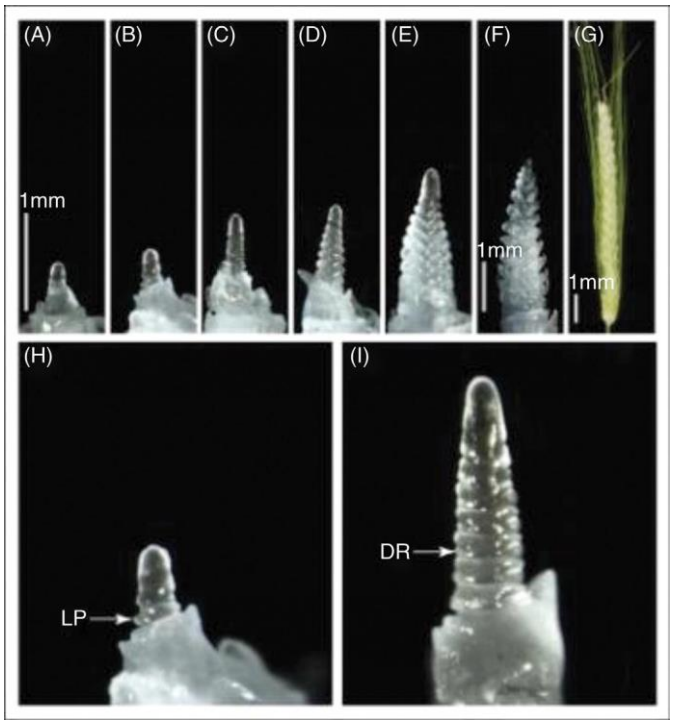


FIGURE 10.2 Phases of cereal shoot apex development taken from (Trevaskis et al., 2007). The shoot apex of barley begin to develop vegetatively and produce leaf primordia (A,B) until inflorescence initiation occurs (C). At this point, floral primordia appear above the leaf primordia, giving rise to distinctive DR along the side of the shoot apex. The floral primordia then differentiate into the floral organs that give rise to the florets (D–G). Anthesis occurs around the time of head emergence (G). Higher magnification images show the morphologic differences between a vegetative shoot apex (H) and a reproductive shoot apex (I). The leaf primordia and DR are indicated by arrows. *Source: Trevaskis et al. (2007); Kirby and Appleyard (1987).*

in cereals,- which could be calibrated and used to monitor a large number of lines (Aitken, 1971). Direct observation of the shoot apex provides accurate information about barley development (Garcia del Moral et al., 2002). It is possible to recognize precise stages of plants (Leather, 2010), however, many researchers describe experimental plants loosely by age (i.e., days), rather than by growth stage.

3 Control of Barley Development

3.1 Environmental and Genetic Controls

Barley development is controlled by genetic factors that modulate the plants' response to environmental cues. In cereals, phenologic adjustments have been mainly due to well-known photoperiod (*Ppd*) and vernalisation (*Vrn*) response genes, as well as earliness *per se* (*Eps or Eam*) loci that affect developmental timing independently (Cockram et al., 2007a; Distelfeld et al., 2009; Campoli and von Korff, 2014). Photoperiod and vernalizing temperatures only affect the rate of development of specific phases, whereas temperature *per se* affects all phases. Many studies have focussed on the genetic control of barley development revealing a complex process with several major genes and many quantitative trait loci (QTL) related to flowering response found on all seven chromosomes of barley (Figure 10.3). The most recent understanding of the cereal flowering genes and their interactions in the flowering pathways are represented by the model in Figure 10.3 and discussed in greater detail in other reviews (Campoli and von Korff, 2014).

A summary of the positions of QTL and flowering time candidate genes from many studies that focused on the genetic control of this trait is shown in Figure 10.4, taken from Campoli and von Korff, 2014 and references within. Other recent genome-wide association scans (GWAS) for preanthesis phases in both short and long photoperiod groups has provided improved power for dissecting the genetic effects on FI and flowering time. In addition to the known major genes that regulate flowering time under field conditions, several other QTLs with varying levels of effect were found to be associated with crop development in recent studies (Alqudah et al., 2014).

Other agronomic factors, such as nutrition and water availability, plant density and radiation (Hall et al., 2014) can modify responses and time to heading. Water stress is the most widely reported, in general speeding up development; however, the effect is relatively small and not constant, often causing greater effect during the later reproductive stages (McMaster et al., 2005). These effects are generally considered of less significance than those of temperature and photoperiod (Garcia del Moral et al., 2002), and will not be discussed here in detail.

3.2 Photoperiod

Barley is classified as a quantitative long day species meaning the progression to flowering is accelerated by increase in photoperiod (Boyd et al., 2003). Genotypes differ in the critical and optimum photoperiods and in the slope of the relationship between rate

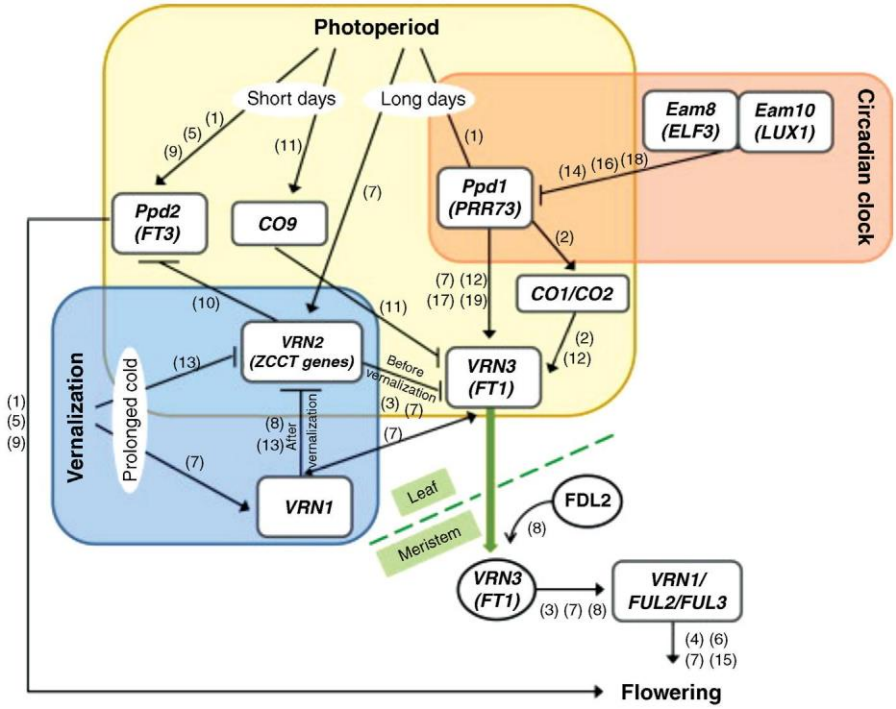


FIGURE 10.3 Model of flowering time control pathways in wheat and barley *(Source: Campoli and von Korff (2014)*. The different external and internal cues are highlighted in different colors. Positive and negative regulatory actions are indicated by arrows and lines with bars, respectively. Boxes indicate genes, while circles indicate proteins. The green arrow shows that the FT1 protein moves from the leaf to the meristem. The figure incorporates different aspects of previously published wheat and barley models. Numbers in brackets indicate literature in which experimental evidences support the model. The numbers referenced here only relate to barley, however the wheat references can be found within Campoli and von Korff (2014). (1) (Laurie et al., 1995) (2) (Turner et al., 2005); (3) (Yan et al., 2006) (5) (Faure et al., 2007) (7) (Hemming et al., 2008) (9) (Kikuchi et al., 2009) (Casao et al., 2011) (12) (Campoli et al., 2012) (14) (Faure et al., 2012); (18) (Campoli et al., 2013).

of development and photoperiod (Roberts et al., 1988). Ppd genes determine the acceleration or delay of flowering time after vernalisation requirements have been satisfied. The most widely known genes related to photoperiod response are *Ppd*-H1 and *Ppd*-H2. *Ppd*-H1 is located on the short arm of chromosome 2H, and is expressed under long days (Laurie et al., 1994, 1995) and has been identified as a Pseudo-Response Regulator-like (PPR-like) gene by positional cloning (Turner et al., 2005). Genotypes can be characterized depending on whether they respond to the influences of a photoperiod over the course of a life cycle, as either photoperiod sensitive or photoperiod insensitive. Two alleles exist and differences between sensitive and insensitive alleles can be found under long photo-period conditions with the dominant allele being sensitive. *Ppd*-H2 is located on the long arm of chromosome 1H and is expressed under short days (Laurie et al., 1995). *HvFT3* is a FT-like (flowering locus T) gene that could be a candidate gene for *Ppd-H2* in barley. The

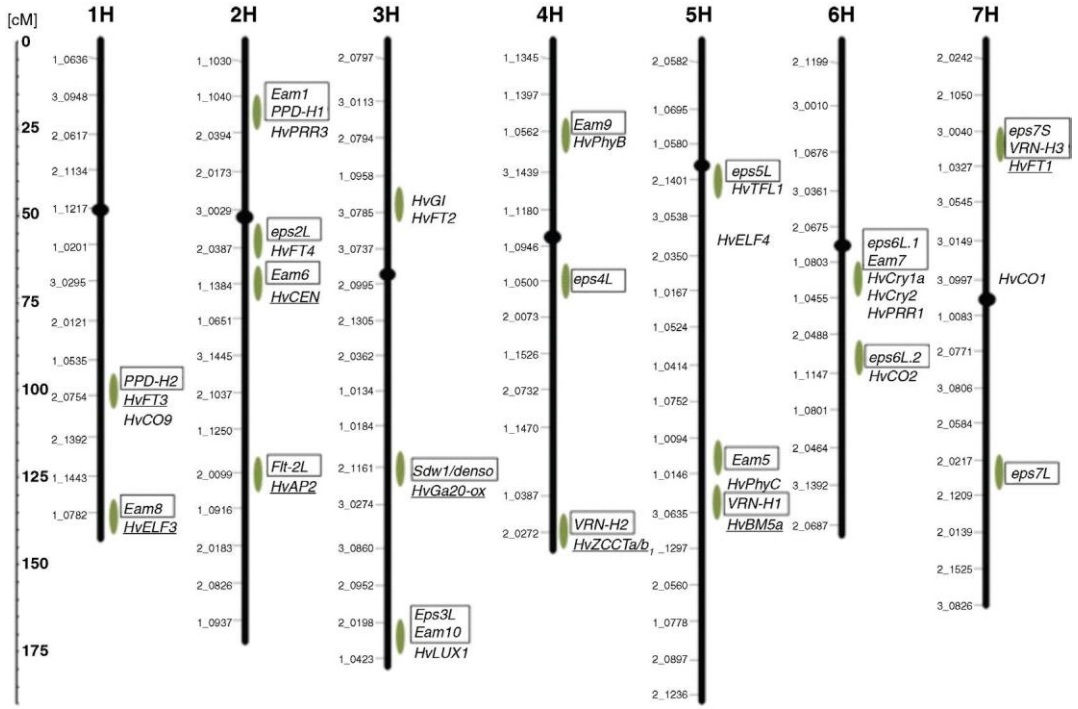


FIGURE 10.4 Consensus map of QTL positions for flowering time in barley taken from (Campoli and von Korff, 2014). Positions of QTL and flowering time candidate genes were projected onto the barley single nucleotide protein (SNP) consensus map of (Muñoz-Amirian et al., 2011). Markers to the left of the chromosomes represent SNP markers. Black ovals indicate the position of the centromeres. Approximate positions of flowering time QTL are indicated by green ovals to the right of the chromosomes. Names of QTL are boxed. Confirmed genes are underlined, whereas suggested candidate genes for QTL are not. The QTL shown are a summary of publications reported in Campoli and von Korff (2014). References for candidate genes are reported in text.

Active allele of *Ppd-H2* is expressed and accelerates flowering under short photoperiod (Laurie et al., 1995; Faure et al., 2007; Kikuchi et al., 2009). Ppd genes have been cloned in barley and functional markers have been developed (Figure 10.3). Many association studies have found that the *Ppd-H1* locus was the major component explaining variation in flowering time among wild barley accessions and domesticated barley landraces and spring barley accessions (Jones et al., 2008).

Plants do not respond to photoperiod during two phases: immediately after germination, namely the juvenile phase or preinductive period, and the post inductive period just prior to heading. Genotypic differences in the duration of these phases are not well under-stood. Photoperiod transfer studies have highlighted that barley can respond to photoperiod during the leaf initiation phase and the spikelet initiation and differentiation phases *independently* (Roberts et al., 1988; Slafer and Rawson, 1994; Miralles and Richards, 2000; Kernich et al., 1995b; Gallagher et al., 1991).

3.3 Vernalisation

Vernalisation is the requirement for a period of exposure to low temperature before the plant apical meristem will transition from vegetative to reproductive development. Vernalisation mainly alters the length of the vegetative phase, and hence FI, which indirectly affects the duration of subsequent preheading phases (González et al., 2002). Genotypes differ in their required duration of exposure to low temperature and in their range of effective vernalizing temperatures (Ritchie, 2002). Vernalisation differences among genotypes range in a quantitative manner between two extremes from zero requirement in "spring" barley cultivars to barley presenting an obligate requirement in the temperature range from 3°C to 12°C (winter types) (Garcia del Moral et al., 2002). Vernalisation response is under strong genetic control. The three major genes responsible for the vernalisation response are: *Vrn-H1* on 5HL (Trevaskis et al., 2003) and *Vrn-H2* on 4HL (Laurie et al., 1995), for which the gene sequences have been identified (Yan et al., 2006), and *Vrn-H3,* which has been mapped on 7HS (Faure et al., 2007).

The gene *VrnH1* is the primary target of vernalisation (Trevaskis et al., 2007), and inter-mediate vernalisation responses are driven mainly by allelic variants of this gene. *VrnH1* is involved in many interactions with other genes along the vernalisation and photoperiod pathway, particularly with *VrnH2* and *VrnH3*. In winter barley types, after adequate cold stimulus, *Vrn-H1* induces the development of the reproductive meristem, whereas *Vrn-H3*, like *Ppd-H1*, is thought to accelerate the late reproductive stages in barley. *Vrn-H2* ne-gates *Ppd-H1* by allowing flowering only after the plant has been exposed to low temperatures (Distelfeld et al., 2009). By comparison, in spring barley the genotypes carrying the dominant alleles Sh do not have a recognized vernalisation requirement and the *Vrn-H2* gene is deleted. Flower development is thus reliant on photoperiod. The classification of genotypes can now be made with molecular markers to characterize alleles for the major vernalisation genes.

3.4 Earliness *per se*

Genotypes of barley differ in flowering date when the requirements of vernalisation and photoperiod have been satisfied (Ellis et al., 1988). Many authors have studied this variation under different contexts; some have implied that the main effect is on the timing of FI and hence duration of the vegetative phase, the basic vegetative period (BVP). Studies taking into account total variation from sowing to heading have described the effect as intrinsic earliness and earliness *per se* (Hay and Elliss, 1998) with the latter generally accepted in the literature (Karsai et al., 2001). Earliness *per se* (Eps) is a complex trait and is less understood than *Ppd* and *Vrn* with smaller, more subtle polygenic effects of the Eps alleles. Genes influencing earliness are distributed throughout the barley genome, and are often reported as QTL with limited information on the underlying genes. The series of early maturity (*Eam)* loci: *eam7, eam8, eam9, and eam10* are located on chromosomes *6HS, 1HL, 4HL, and 3HL*, respectively, and recently the *eam* loci *eam6*, *eam8*, and *eam10* have been cloned (Campoli et al., 2013; Faure et al., 2012; Zakhrabekova et al., 2012).

The effect of the ambient temperature is considered to universally affect all genotypes and every developmental phase, from emergence to maturity (Garcia del Moral et al., 2002). However, Eps is also influenced by temperature which is largely determined by the gene and allele considered (Slafer and Rawson, 1995). Further evidence from recent studies with the gene *Eps-Am1* (Lewis et al., 2008) support findings by other authors showing that, within the term earliness *per se*, there are large genotypic differences in the response to nonvernalizing temperatures or temperature *per se* (Ellis et al., 1988; Slafer, 1996) and responses may not be constant throughout development, meaning that the duration of phases differs in sensitivity (Slafer, 1996; Gómez-Macpherson and Richards, 1997). Differences in Eps are often not explained by thermal time, calendar time, nor different temperature conditions as genotypes also can differ in their base and optimum temperatures in each phase (Slafer and Rawson, 1995; Garcia del Moral et al., 2002).

3.5 Basic Vegetative Period

The term basic vegetative period (BVP) has been adopted in Australia among breeders and physiologists (Boyd et al., 2003) and has been used to define the period in which plants re-main insensitive to the otherwise inductive effects of photoperiod. BVP is measured under optimal (saturated) photoperiod and is often based on surrogate measures of FI including the timing to awn peep/anthesis or with main stem leaf number. The measurement assumes that the timing of anthesis strongly reflects the timing of FI and that photoperiod responses are not independent of one another throughout any of the other sub phases of development. This may not be the case as previously suggested, as there is lack of a clear correlation between early development and heading date in some circumstances (Vanoosterom and Acevedo, 1992). Furthermore, heading date is subject to many con-founding differences in development rates with respect to other environmental stimuli. Therefore, as suggested by (Boyd et al., 2003), the term BVP is only appropriate for measures of duration from sowing to FI.

4 Phenology and Adaptation: Matching Crop Phenology to Growing Conditions in Australia

Crop phenology matched with availability of resources and avoidance of stress events during grain filling (Slafer et al., 2005; Reynolds et al., 2009) is the most important factor influencing yield and crop adaptation to particular environments (Richards, 1991). In Australia, climatic conditions in the temperate cereal production areas define the critical periods for sowing and for the phenologic events which follow, such as the transition from the vegetative phase to reproductive and flowering time. The identification of genes that influence individual phases of development would allow for further fine-tuning to different environmental demands to increase yield. This section of the review will focus on developmental variations in Australian barley, and their contribution toward and potential to improve yield. As information on barley is scarce, studies on other species, chiefly wheat, will be considered.

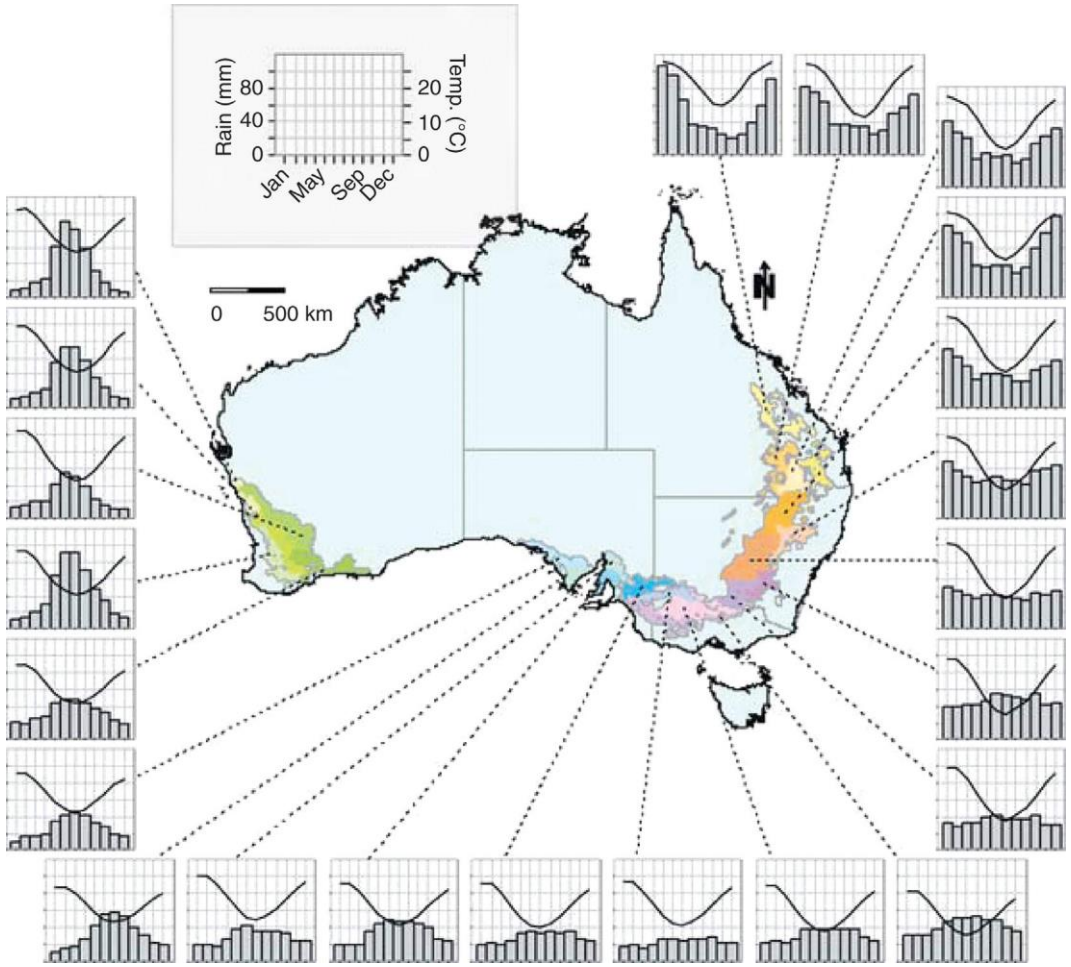


FIGURE 10.5 Map of monthly cumulative rainfall (bars) and monthly average temperature (solid line) in the 22 regions of the Australian wheat belt for the period 1889–2011. Data are averaged across locations within each region (Table 10.1 and Figure 10.1). Scales are given in the shaded box at the top of the figure. *Source: Chenu et al. (2013).*

4.1 Growing Regions and Seasonal Conditions

The climatic conditions of the temperate barley production areas in Australia are characterised by hot and dry summers and cool moist winters (Figure 10.5). Northern New South Wales and parts of Southern Queensland are under a summer dominant rainfall pattern, whereas Western Australia and South Australia are winter dominant and the remaining areas of Southern New South Wales and Victoria share an equal distribution of summer and winter rainfall (McKenzie, 2004). Despite the differing rainfall patterns, barley is pre-dominantly grown in dry-land farming systems reliant on stored and/or in-crop rainfall in regions of annual rainfall between 300 mm and 600 mm. Spring barley types are typically sown in late autumn (May) or early winter (June) and grow through to spring (October)

and are harvested in early summer. The variability in growing season rainfall (April–October) is high within regions and between seasons, ranging from 100 mm ~ 550 mm, de-pending on the region, and is typically more reliable during the winter and early spring months, declining during the later parts of spring (Fischer, 2009; Chenu et al., 2013).

4.2 Agronomic and Breeding Significance

Sowing conditions are usually favourable for crop establishment and early vegetative growth, with growing temperatures tapering during winter but mild enough so that cold damage is uncommon. Growth conditions rapidly improve as spring approaches through-out the period of stem elongation (SE) and heading. During the grain development phase, conditions begin to deteriorate and become unfavourable, as demand for soil moisture of-ten exceeds supply.

Temperatures, photoperiod, and evaporation follow a different trend with temperatures relatively warm with longer days in April and decreasing from the opening of the season until mid ~ late winter, and increasing thereafter. Frost events are common in late winter and early spring along with hot and dry spells during late spring. The optimum flowering window (Figure 10.6) is determined by the competing demands of frost avoidance during flowering and completing grain fill prior to the high temperatures and dry spells commonly experienced in Australian springs (Shackley, 2000), all of which significantly reduce grain yield (Richards, 1991). Developing varieties with a flowering date that optimally matches the growing season is therefore a primary objective of breeding programs (Boyd et al., 2003). Based on model experimentation, the most common and severe water stress starts about 400 Cd before flowering in wheat (Chenu et al., 2013), in coincidence with the critical period of crop development. These patterns are yet to be determined for barley, although they are likely to be similar to wheat.

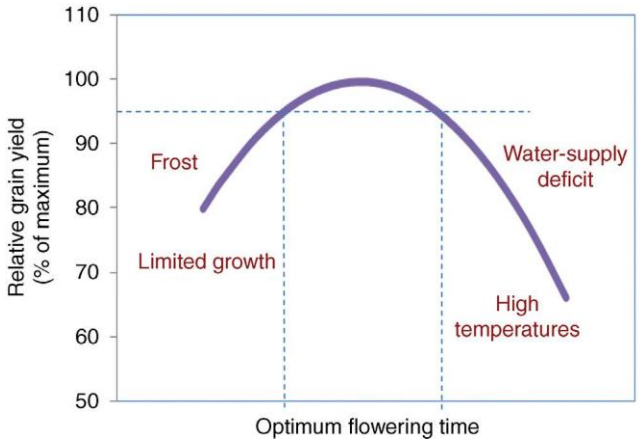


FIGURE 10.6 Grain yield response to flowering time: the concept of the "flowering window." *Adapted from: Anderson and Garlinge (2003).*

4.3 Developmental Variation in Australian and Introduced Genotypes

The best-adapted barley cultivars for Australian low-medium rainfall environments are early-maturing spring types, which exhibit a relatively high photoperiod response. Longer season types have been favoured in the higher rainfall zones of Tasmania and southern Victoria as well as in small regions of WA and southern NSW (Young and Elliott, 1994; Paynter et al., 2001; Boyd et al., 2003).

Most adapted Australian cultivars and spring types introduced from Europe, Canada, and Japan have either no or a very minimal *vernalisation* requirement, with the exception of Ulandra and, more recently, Urambie and Yambla, which were bred for grazing purposes and may also be useful for very early sowing opportunities. Based on heading dates under short and long days, almost all commercial spring releases in Australia possess a relatively short duration to heading and very strong response to increase in photoperiod; a longer vegetative growth would effectively extend grain fill toward conditions of declining rainfall, increasing temperature, and high evaporative demand (Boyd et al., 2003).

By comparison, most spring types released in Europe and North America exhibit long durations to heading but minimal responses to extended photoperiod (Boyd et al., 2003). Ren et al. (2010) noted large differences in heading dates among genotypes between Australian and Southeast Asian environments, despite similar latitudes. These studies indicated there were genes conditioned by temperature differences between the locations and suggested that variation in duration to heading existed for reasons other than vernalisation and photoperiod. This may be an important adaptive trait for Australian cultivars. Temperature effects could in fact be equal to or even greater than some of the most extreme responses re-corded for vernalisation or photoperiod (Boyd et al., 2003) when grown under milder winter growing conditions. Landraces with erect plant type originating from Jordanian landscapes characterized by relatively mild winters and terminal drought stress were identified as having potentially important characteristics for Australian conditions. The combination of an appropriate earliness and good early vigour to achieve ground cover in spring resembled spring barley types required for Australian environments and was a key factor in attaining high yields in Mediterranean stressful environments (Vanoosterom and Acevedo, 1992).

4.4 Mapping Studies of Phenologic Variation

There are many barley populations that segregate for major phenology genes (Figure 10.4) and there are many QTL for heading date whose positions do not appear to coincide with the main phenology genes (Borras-Gelonch et al., 2010). The genetic analysis of mapping populations of Australia identified 16 QTL with contributions ranging from <10% to >50% of the variation recorded in heading date. QTL associated with chromosome 2 were of major and consistent influence, one being associated with 2HS (near Ppd H1) and the other near the centromeric region 2HC (Boyd et al., 2003). In the Sloop × Halcyon mapping population, there were several QTL governing flowering time which were located near the centromeric region of 2H and on 5H (Read et al., 2003). Despite particular *Vrn* and *Ppd*- alleles- appearing more frequently in some geographical areas, variation among genotypes

has also been found within regions. Therefore, it is equally likely that different combinations of developmental gene alleles would be found in successful genotypes well adapted to particular regions. This reinforces the notion that several other genes and their combined effects may be influential in the control of flowering time (Cockram et al., 2007b).

4.5 Linking Gene Sequence Information With Field Performance

Genetic resources previously identified can begin to be used to assess the functional variation of phenology gene alleles, gene–gene, and gene–environment interactions. The obvious next step is to link gene sequence information with field performance to accelerate genetic gains within breeding through marker-assisted selection, particularly by targeting favourable phenology gene combinations. Utilizing multidisciplinary genomics knowledge, the identification of potential gene targets will make it possible to develop molecular ideotypes to meet future breeding requirements (Sreenivasulu and Schnurbusch, 2012). Genomics-based approaches provide better access to agronomic desirable alleles present at QTL and genes affecting crop responses (Tondelli et al., 2014). If gene sequence information is to be linked with field performance, there is a need to better understand the role that phenology genes play in the determination of yield.

Improved adaptation and grain yield under Mediterranean conditions depend to a remarkable extent on phenology, however, not all phenology genes affect grain yield to the same extent or in the same manner. Most studies on the genetic control of development have focused on total time to heading and yield using mapping populations derived from wide crosses between materials with major differences in phenology genes, yield, and flowering time responses. This approach may be misleading, as it is not representative of elite varieties and it means that any other trait or component of the developmental pattern, that might be associated with yield but whose effect may be relatively small, may not be uncovered. Laurie et al. (1994) demonstrated the *Ppd-H1* locus can cause pleiotropic effects on plant height and yield components as a result of the effect on flowering time, and Coventry et al. (2003) found associations of *Ppd-H1, eps2, Shj (4HL), Sh2 (5HL), and eps 7HS (7HS)* loci with grain weight and size QTL in barley. The grain-yield link with phenology was better explained after considering epistatic interactions with the role of *VrnH1* in the determination of grain yield being intensified by its interaction with other QTL (Mansour et al., 2014). This means that even in highly elite material, there is room for improvement and fine-tuning of some of the main adaptation genes and, equally, the combination of phenology genes are critically important.

5 Manipulating Developmental Phases for Further Yield Improvement

Optimizing the partitioning of time to heading by manipulating vegetative and reproductive phases independently without modifying total time to heading has been proposed for further yield improvement (Appleyard et al., 1982; Kitchen and Rasmusson, 1983; Slafer et al., 2005; Borras et al., 2009; Borras-Gelonch et al., 2012). Most breeding programs have

focused on improved adaptation by empirical selection for grain yield and heading date. This approach implies indirect selection for changes in preanthesis developmental phases. Flowering time is a key adaptive trait and is often already optimized in any breeding program (Slafer et al., 2005) and therefore, total time to flowering is well adjusted for a particular environment (Isidro et al., 2011), particularly in Australia where the optimum flowering window is relatively narrow (Boyd et al., 2003). There may be little room for improving barley adaptability and yield by further adjustments in time to heading (Slafer et al., 2005).

5.1 Improving Grain Number

Given that grains per meter square has been the primary determinant for increases in grain yield in modern cultivars (Peltonen-Sainio et al., 2007) focusing on fine adjustment of the phenologic traits that universally influence grain number and identifying their genetic basis may prove effective. Analysing yield variation and yield components with genotype and environments has been the traditional approach, however, more recently in wheat the relationships between yield or grains per meter square and their components have been demonstrated to change depending on the scale and nature of the variation (Slafer et al., 2014). Importantly, the conclusions reached by this study found that any yield component is able to function as a fine-tuning mechanism, and that the number of grains/ m^2 could be responsible for coarse regulation of yield in which the number of spikes/m^2 was mainly driven by environmental regulation factors, whereas the number of grains per spike were driven by genotypic factors. (Slafer et al., 2014). Maximum yield potential in barley occurs at the awn primordia (AP) stage when maximum floret number is set (Kirby and Appleyard, 1987). The period from 10 days after SE to 10 days before flag leaf emergence has been defined as the most critical externally identifiable period for setting grains (Arisnabarreta and Miralles, 2008), and more recently, the period from AP to heading as the most critical period for yield in barley as it is directly related to spikelet reduction (survival) and grain yield per main spike (Alqudah and Schnurbusch, 2014) (Figure 10.7).

5.2 Manipulating the Critical Period

Extending the length of the critical period may increase the number of fertile florets and yield by increasing assimilate translocation and acquisition by the spikes (Slafer and Raw-son, 1994; Kernich et al., 1997; Miralles et al., 2000). In wheat this is due to less competition between stem and spike determining heavier spikes at flowering and allowing a sustained floret development (Hawkesford et al., 2013). There is a strong link between photoperiod sensitivity, the duration of spike development, and spike fertility (Miralles and Slafer, 2007). This is well represented by the model diagram in Figure 10.8, developed by Sadras and Slafer (2012), in that during the critical period, there is an (1) overproduction of florets and only a proportion of them remain fertile (González et al., 2011) and depending on photoperiod response, it (2) reduces floret number, (3) by shortening the duration of spike growth, and hence reduces spike dry weight at anthesis. Factors other than photoperiod, such as resource availability, (4) also influence the relationship between floret mortality and spike dry weight (Sadras and Slafer, 2012). This model also raises important questions about

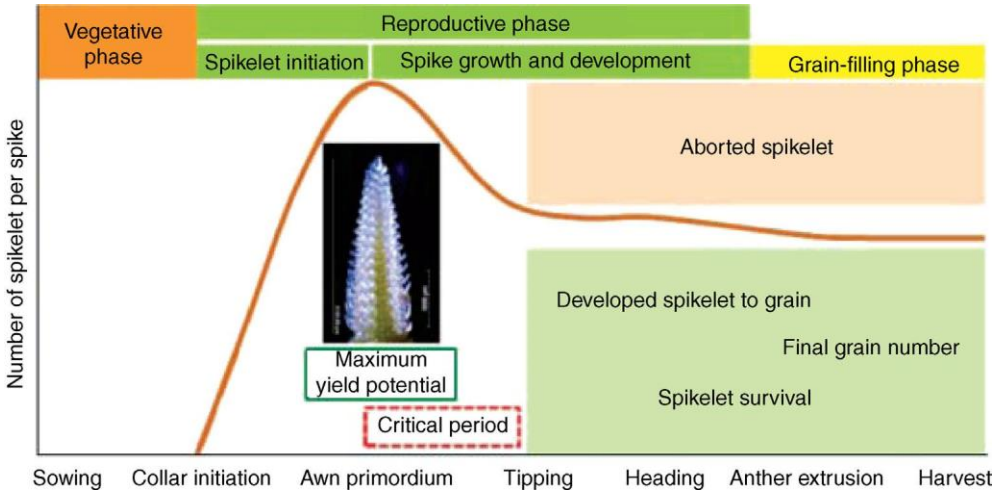


FIGURE 10.7 General trend of spikelet numbers per spike with relation to growing degree days and phenologic stages and phases of development. *Source: Alqudah and Schnurbusch (2014*

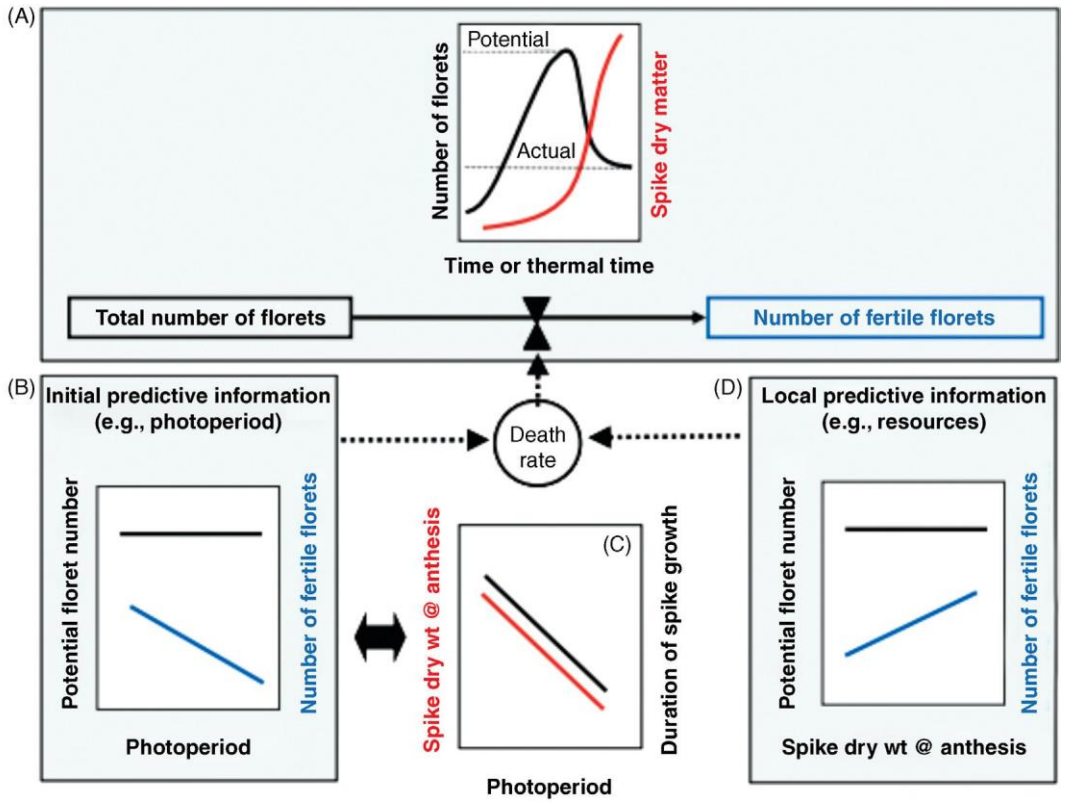


FIGURE 10.8 Modelled framework of plant responses to environmental factors highlighting the modulation of floret number and spike growth in wheat. *Source: Sadras and Slafer (2012).*

potential trade-offs, particularly with reference to grain number and grain-size dynamics; if manipulating preflowering phases increases grain number, this may reduce grain size.

5.3 Genetic Control of Preheading Phases

There is evidence of genetic control of duration of the preanthesis phases (Sreenivasulu and Schnurbusch, 2012), particularly during the late reproductive phase in barley, even within genotypes that possess similar time to heading (Appleyard et al., 1982; Kitchen and Rasmusson, 1983; Kernich et al., 1995a, 1997; Whitechurch et al., 2007; Borras-Gelonch et al., 2010). Without compromising spikelet initiation, reducing the time to FI is possible if independent variability were identified as in Kitchen and Rasmusson (1983). Responses to photoperiod and temperature also differ between preheading phases, and responses during the spikelet differentiation phase can be even greater than in previous phases (Roberts et al., 1988; Slafer and Rawson, 1994; Miralles et al., 2000; González et al., 2005). Spikelet survival can be negatively affected under long-day conditions due to a shortened SE peri-od (Kernich et al., 1996) Despite a similar time to anthesis, the cultivar Schooner achieved greater spikelet survival compared to Weeah, due to an extended spike growth phase (Kernich et al., 1997) (Figure 10.9, Table 10.1).

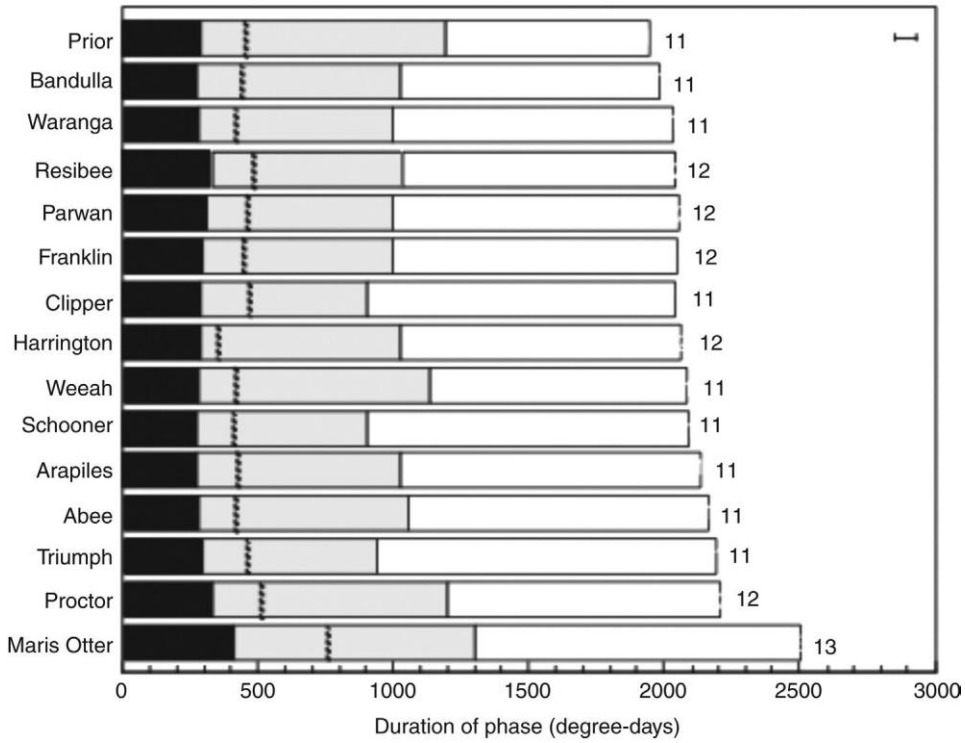


FIGURE 10.9 Duration of the leaf initiation (dark shading), spikelet initiation (gray shading), and spikelet growth (open bars) phases for cultivars grown in a glasshouse. Error bar (l.s.d.) indicates difference ($P < 0.05$) in time to anthesis only. *Source: Kernich et al. (1997).*

Table 10.1 Preanthesis Development in Barley, Maximum Number of Spikelet Primordia, Final Spikelet Number, and Spikelet Abortion for Barley Cultivars

Cultivar	Max. No. of Spikelet Primordia	Final Spikelet No.	Spikelet Abortion (%)
Abee	38.3	23.9	38
Arapiles	40.5	32.4	20
Bandulla	41.5	24.4	41
Clipper	38.8	29.7	23
Franklin	39.8	31.3	21
Harrington	38.0	31.7	17
Maris Otter	36.8	33.1	10
Parwan	38.0	27.0	29
Prior	41.0	30.5	26
Proctor	41.5	36.6	12
Resibee	37.0	30.6	17
Schooner	38.8	32.1	17
Triumph	40.5	25.1	38
Waranga	39.0	27.1	31
Weeah	42.3	25.9	39
1.s.d. (P = 0.05)	1.3	1.3	–

Source: Kernich et al. (1997).

The duration of the critical period in barley could potentially be manipulated by major developmental genes such as photoperiod sensitivity genes, and/or earliness *per se* genes. There are several studies comparing wheat lines differing in *Ppd* alleles showing differences between genotypes in the duration of preheading phases and in the response to photo-period of each sub phase (González et al., 2005) although García et al. (2011) showed that the phenotypic variability observed for a longer critical period in lines with similar cycle to flowering was not clearly associated with major adaptation genes evaluated, suggesting that other minor genes could be associated. A better understanding of the genetic control of these preanthesis phases could identify opportunities to improve grain yield without changing the total cycle length (Garcia del Moral et al., 2002) and may help to improve the knowledge of phenologic traits driving adaptability (Limin et al., 2007).

5.4 Mapping Studies of Preanthesis Phases

The most recent studies attempting to identify the genetic basis of the preanthesis phases in barley were conducted in the Steptoe × Morex population (Borras-Gelonch et al., 2012), which is known to segregate for major phenology genes, and in the barley population Henni × Meltan (H × M), which has a much narrower genetic base for phenologic traits (Bor-ras et al., 2009). Independent genotypic variability in the duration of spikelet initiation (SI) and SE was found in each population, and several minor QTL with additive effects for these differences were identified that had little to no effect in total time to flowering. QTL between LS and SE in H × M were not related to major genes, whereas in the S × M population, major flowering genes were responsible for part of the differences in the ratio SE/LS,

namely *Ppd–H2* and to the *Eam6* loci. This Eam6 locus has major effects on flowering time in Australian environments and has been associated with variation in the duration of the basic vegetative period and yield component traits, such as kernel weight, plant height, and peduncle length (Boyd et al., 2003). In spite of significant genotype × environment and QTL × E effects for both LS and SE, differences between genotypes in the ratio SE/LS were well-maintained across environments (Borras-Gelonch et al., 2010, 2012). Most studies have compared a wide range of photoperiod (up to 18 h) and temperature conditions and may give an unrealistic idea of G × E interactions of most growing conditions for the partitioning of time to heading. Finding independent, albeit small, QTL control of these phases in the Henni × Meltan population was a relevant result as the variability in this population was limited (Borras et al., 2009) and reflects more of what occurs in modern breeding programs.

6 Conclusions

The ideas presented here are important for identifying new opportunities to improve yield. This review has presented a broad description of the major developmental stages of barley and the important relationships between crop phenology and yield determination with reference to Australian conditions. It is clear that there is significant phenologic variation in cultivars adapted to Australian conditions based on classical and molecular studies. However, the current physiologic understanding of the link between phenology and yield determination in well-adapted varieties under field conditions is very limited. The most favourable combinations of genes conferring adaptation remain relatively unknown and there is a need to link gene sequencing data to phenotype and field performance. Manipulating duration of the developmental phases prior to heading provides real potential for further improvements in yield. A collaborative approach combining both fundamental physiological research and new molecular tools to disentangle the key phenologic traits driving yield may offer new insights into manipulating phenology for further yield improvement.

References

Abeledo, L.G., Calderini, D.F., Slafer, G.A., 2002. Physiological changes associated with genetic improvement of grain yield in barley. Barley: Recent Advances From Molecular Biology to Agronomy of Yield and Quality. Food Product Press, New York, pp. 361–386.

Aitken, Y., 1971. Non-destructive methods for estimation of flower initiation in subterranean clover and cereals. J. Aust. Inst. Agric. Sci. 37, 57–60.

Alqudah, A.M., Schnurbusch, T., 2014. Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Funct. Plant. Biol. 41, 424–436.

Alqudah, A.M., Sharma, R., Pasam, R.K., Graner, A., Kilian, B., Schnurbusch, T., 2014. Genetic dissection of photoperiod response based on GWAS of pre-anthesis phase duration in spring barley. PLoS ONE 9, e113120.

Anderson, W.K., Garlinge, J.R., 2003. The wheat book. Principles and Practice. Department of Agriculture, Western Australia.

Appleyard, M., Kirby, E.J.M., Fellowes, G., 1982. Relationships between the duration of phases in the pre-anthesis life cycle of spring barley. Aust. J. Agric. Res. 33, 917–925.

Araus, J.L., Slafer, G.A., Royo, C., Serret, M.D., 2008. Breeding for yield potential and stress adaptation in cereals. Crit. Rev. Plant Sci. 27, 377–412.

Arisnabarreta, S., Miralles, D.J., 2008. Critical period for grain number establishment of near isogenic lines of two- and six-rowed barley. Field Crop Res. 107, 196–202.

Borras, G., Romagosa, I., van Eeuwijk, F., Slafer, G.A., 2009. Genetic variability in duration of pre-heading phases and relationships with leaf appearance and tillering dynamics in a barley population. Field Crop Res. 113, 95–104.

Borras-Gelonch, G., Slafer, G.A., Casas, A.M., van Eeuwijk, F., Romagosa, I., 2010. Genetic control of pre-heading phases and other traits related to development in a double-haploid barley (*Hordeum vulgare* L.) population. Field Crop Res. 119, 36–47.

Borras-Gelonch, G., Denti, M., Thomas, W.T.B., Romagosa, I., 2012. Genetic control of pre-heading phases in the Steptoe × Morex barley population under different conditions of photoperiod and temperature. Euphytica 183, 303–321.

Boyd, W.J.R., Li, C.D., Grime, C.R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Kamali, M.R.J., Barr, A.R., Moody, D.B., Lance, R.C.M., Logue, S.J., Raman, H., Read, B.J., 2003. Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (*Hordeum vulgare* L.) genotypes grown over a mild winter growing season. Aust. J. Agric. Res. 54, 1277–1301.

Campoli, C., von Korff, M., 2014. Genetic control of reproductive development in temperate cereals. Advances in Botanical Research. Academic Press, Oxford, UK, pp. 131–158 (Chapter 5).

Campoli, C., Drosse, B., Searle, I., Coupland, G., Von Korff, M., 2012. Functional characterisation of HvCO1, the barley (*Hordeum vulgare*) flowering time ortholog of CONSTANS. Plant J. 69, 868–880.

Campoli, C., Pankin, A., Drosse, B., Casao, C.M., Davis, S.J., Von Korff, M., 2013. *HvLUX1* is a candidate gene underlying the early maturity 10 locus in barley: phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytol. 199, 1045–1059.

Casao, M.C., Igartua, E., Karsai, I., Lasa, J.M., Gracia, M.P., Casas, A.M., 2011. Expression analysis of vernalisation and day-length response genes in barley (*Hordeum vulgare* L.) indicates that VRNH2 is a repressor of PPDH2 (HvFT3) under long days. J. Exp. Bot. 62, 1939–1949.

Chenu, K., Deihimfard, R., Chapman, S.C., 2013. Large-scale characterization of drought pattern: a continent-wide modelling approach applied to the Australian wheat belt – spatial and temporal trends. New Phytol. 198, 801–820.

Cockram, J., Chiapparino, E., Taylor, S.A., Stamati, K., Donini, P., Laurie, D.A., O'Sullivan, D.M., 2007a. Haplotype analysis of vernalisation loci in European barley germplasm reveals novel *VRN-H1* alleles and a predominant winter *VRN-H1*/*VRN-H2* multi-locus haplotype. Theor. Appl. Genet. 115, 993– 1001.

Cockram, J., Jones, H., Leigh, F.J., O'Sullivan, D., Powell, W., Laurie, D.A., Greenland, A.J., 2007b. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. J. Exp. Bot. 58, 1231–1244.

Coventry, S.J., Barr, A.R., Eglinton, J.K., McDonald, G.K., 2003. The determinants and genome locations influencing grain weight and size in barley (*Hordeum vulgare* L.). Aust. J. Agric. Res. 54, 1103–1115.

Distelfeld, A., Li, C., Dubcovsky, J., 2009. Regulation of flowering in temperate cereals. Curr. Opin. Plant Biol. 12, 178–184.

Ellis, R.H., Roberts, E.H., Summerfield, R.J., Cooper, J.P., 1988. Environmental control of flowering in barley (*Hordeum vulgare* L.) II. Rate of development as a function of temperature and photoperiod and its modification by low-temperature vernalisation. Ann. Bot. 62, 145–158.

Faure, S., Higgins, J., Turner, A., Laurie, D.A., 2007. The FLOWERING LOCUS T-like gene family in barley (*Hordeum vulgare*). Genetics 176, 599–609.

Faure, S., Turner, A.S., Gruszka, D., Christodoulou, V., Davis, S.J., Von Korff, M., Laurie, D.A., 2012. Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (*Hordeum vulgare*) to short growing seasons. Proc. Natl. Acad. Sci. USA 109, 8328–8333.

Fischer, R.A., 2007. Understanding the physiological basis of yield potential in wheat. J. Agric. Sci. 145, 99–113.

Fischer, R.A., 2009. Farming systems of Australia: exploiting the synergy between genetic improvement and agronomy. Crop Physiology. Academic Press, San Diego, pp. 22–54 (Chapter 2).

Gallagher, L.W., Soliman, K.M., Vivar, H., 1991. Interactions among loci conferring photoperiod insensitivity for heading in spring barley. Crop Sci. 31, 256–261.

Garcia del Moral, L.G., Miralles, D.J., Slafer, G.A., 2002. Initiation and appearance of vegetative and reproductive structures throughout barley development. Barley Science: Recent Advances From Molecular Biology to Agronomy of Yield and Quality. Food Product Press, New York, pp. 243–267.

García, G.A., Serrago, R.A., Appendino, M.L., Lombardo, L.A., Vanzetti, L.S., Helguera, M., Miralles, D.J., 2011. Variability of duration of pre-anthesis phases as a strategy for increasing wheat grain yield. Field Crop Res. 124, 408–416.

Gómez-Macpherson, H., Richards, R.A., 1997. Effect of early sowing on development in wheat isolines differing in vernalisation and photoperiod requirements. Field Crop Res. 54, 91–107.

González, F.G., Slafer, G.A., Miralles, D.J., 2002. Vernalisation and photoperiod responses in wheat pre-flowering reproductive phases. Field Crop Res. 74, 183–195.

González, F.G., Slafer, G.A., Miralles, D.J., 2005. Floret development and survival in wheat plants exposed to contrasting photoperiod and radiation environments during stem elongation. Funct. Plant Biol. 32, 189–197.

González, F.G., Miralles, D.J., Slafer, G.A., 2011. Wheat floret survival as related to pre-anthesis spike growth. J. Exp. Bot. 62, 4889–4901.

Hall, A.J., Savin, R., Slafer, G.A., 2014. Is time to flowering in wheat and barley influenced by nitrogen? A critical appraisal of recent published reports. Eur. J. Agron. 54, 40–46.

Hamid, Z.A., Grafius, J.E., 1978. Developmental allometry and its implication to grain yield in barley. Crop Sci. 18, 83–86.

Haun, J.R., 1973. Visual quantification of wheat development. Agron. J. 65 (1), 116–119.

Hawkesford, M.J., Araus, J.L., Park, R., Calderini, D., Miralles, D., Shen, T., Zhang, J., Parry, M.A.J., 2013. Prospects of doubling global wheat yields. Food Energy Secur. 2, 34–48.

Hay, R.K.M., Elliss, R.P., 1998. The control of flowering in wheat and barley: what recent advances in molecular genetics can reveal. Ann. Bot. 82, 541–554.

Hemming, M.N., Peacock, W.J., Dennis, E.S., Trevaskis, B., 2008. Low-temperature and day length cues are integrated to regulate Flowering Locus T in barley. Plant Physiol. 147, 355–366.

Isidro, J., Álvaro, F., Royo, C., Villegas, D., Miralles, D.J., García del Moral, L.F., 2011. Changes in duration of developmental phases of durum wheat caused by breeding in Spain and Italy during the 20th century and its impact on yield. Ann. Bot. 107, 1355–1366.

Jones, H., Leigh, F.J., Mackay, I., Bower, M.A., Smith, L.M.J., Charles, M.P., Jones, G., Jones, M.K., Brown, T.A., Powell, W., 2008. Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the Fertile Crescent. Mol. Biol. Evol. 25, 2211–2219.

Karsai, I., Mészáros, K., Láng, L., Hayes, P.M., Bedö, Z., 2001. Multivariate analysis of traits determining adaptation in cultivated barley. Plant Breed. 120, 217–222.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1995a. Variation in developmental patterns of wild barley (*Hordeum spontaneum* L.) and cultivated barley (*Hordeum vulgare* L.). Euphytica 82, 105–115.

Kernich, G.C., Slafer, G.A., Halloran, G.M., 1995b. Barley development as affected by rate of change of photoperiod. J. Agric. Sci. 124, 379–388.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1996. Constant and interchanged photoperiod effects on the rate of development in barley (*Hordeum vulgare*). Aust. J. Plant Physiol. 23, 489–496.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1997. Variation in duration of pre-anthesis phases of development in barley (*Hordeum vulgare*). Aust. J. Agric. Res. 48, 59–66.

Kikuchi, R., Kawahigashi, H., Ando, T., Tonooka, T., Handa, H., 2009. Molecular and functional characterization of pebp genes in barley reveal the diversification of their roles in flowering. Plant Physiol. 149, 1341–1353.

Kirby, E.J.M., 1988. Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. Field Crop Res. 18, 127–140.

Kirby, E., Appleyard, M., 1987. Cereal Development Guide. NAC Cereal Unit, Stoneleigh, UK.

Kitchen, B.M., Rasmusson, D.C., 1983. Duration and inheritance of leaf initiation, spike initiation and spike growth in barley. Crop Sci. 23, 939–943.

Laurie, D.A., Pratchett, N., Bezant, J.H., Snape, J.W., 1994. Genetic analysis of photoperiod response gene on the short arm of chromosome 2 (2H0 of *Hordeum vulgare* (barley). Heredity 72, 619–627.

Laurie, D.A., Pratchett, N., Snape, J.W., Bezant, J.H., 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38, 575–585.

Leather, S.R., 2010. Precise knowledge of plant growth stages enhances applied and pure research. Ann. Appl. Biol. 157, 159–161.

Lewis, S., Faricelli, M.E., Appendino, M.L., Valarik, M., Dubcovsky, J., 2008. The chromosome region including the earliness *per se* locus Eps-A(m)1 affects the duration of early developmental phases and spikelet number in diploid wheat. J. Exp. Bot. 59, 3595–3607.

Limin, A., Corey, A., Hayes, P., Fowler, D.B., 2007. Low-temperature acclimation of barley cultivars used as parents in mapping populations: response to photoperiod, vernalisation and phenological development. Planta 226, 139–146.

Mansour, E., Casas, A.M., Gracia, M.P., Molina-Cano, J.L., Moralejo, M., Cattivelli, L., Thomas, W.T.B., Igartua, E., 2014. Quantitative trait loci for agronomic traits in an elite barley population for Mediterranean conditions. Mol. Breed. 33, 249–265.

McKenzie, N., 2004. Australian Soils and Landscapes an Illustrated Compendium. CSIRO Pub., Collingwood, Victoria.

McMaster, G.S., Wilhelm, W.W., Frank, A.B., 2005. Developmental sequences for simulating crop phenology for water-limiting conditions. Aust. J. Agric. Res. 56, 1277–1288.

Miralles, D.J., Richards, R.A., 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Ann. Bot. 85, 655–663.

Miralles, D.J., Slafer, G.A., 2007. Sink limitations to yield in wheat: how could it be reduced? J. Agric. Sci. 145, 139–149.

Miralles, D.J., Richards, R.A., Slafer, G.A., 2000. Duration of the stem elongation period influences the number of fertile florets in wheat and barley. Aust. J. Plant Physiol. 27, 931–940.

Muñoz-Amatriaín, M., Moscou, M.J., Bhat, P.R., Svensson, J.T., Bartoš, J., Suchánková, P., Šimková, H., Endo, T.R., Fenton, R.D., Lonardi, S., Castillo, A.M., Chao, S., Cistué, L., Cuesta-Marcos, A., Forrest, K.L., Hayden, M.J., Hayes, P.M., Horsley, R.D., Makoto, K., Moody, D., Sato, K., Vallés, M.P., Wulff, B.B.H., Muehlbauer, G.J., Doležel, J., Close, T.J., 2011. An improved consensus linkage map of barley based on flow-sorted chromosomes and single nucleotide polymorphism markers. Plant Genome 4, 238–249.

Paynter, B.H., Juskiw, P.E., Helm, J.H., 2001. Phenological development in two-row spring barley when grown in a long-day (Alberta, Canada) and a short-day (Western Australia, Australia) environment. Can. J. Plant Sci. 81, 621–629.

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

Read, B.J., Raman, H., McMichael, G., Chalmers, K.J., Ablett, G.A., Platz, G.J., Raman, R., Genger, R.K.,Boyd, W.J.R., Li, C.D., Grime, C.R., Park, R.F., Wallwork, H., Prangnell, R., Lance, R.C.M., 2003. Mapping and QTL analysis of the barley population Sloop & Halcyon. Aust. J. Agric. Res. 54, 1145–1153.

Ren, X.F., Li, C.D., Boyd, W.J.R., Westcott, S., Grime, C.R., Sun, D.F., Lance, R., 2010. QTLs and their interaction determining different heading dates of barley in Australia and China. Crop Pasture Sci. 61, 145–152.

Reynolds, M., Foulkes, M.J., Slafer, G.A., Berry, P., Parry, M.A.J., Snape, J.W., Angus, W.J., 2009. Raising yield potential in wheat. J. Exp. Bot. 60, 1899–1918.

Richards, R.A., 1991. Crop improvement for temperate Australia, future opportunities. Field Crop Res. 26, 141–169.

Ritchie, J.T., 2002. Computer simulation of barley growth, development, and yield. Barley Science. NY Food Products Pres, New York, pp. 243–268.

Roberts, E.H., Summerfield, R.J., Cooper, J.P., Ellis, R.H., 1988. Environmental control of flowering in barley (*Hordeum vulgare* L). 1. Photoperiod limits to long day response, photoperiod insensitive phases and effects of low temperature and short day vernalisation. Ann. Bot. 62, 127–144.

Sadras, V.O., Denison, R.F., 2009. Do plant parts compete for resources? An evolutionary viewpoint. New Phytol. 183, 565–574.

Sadras, V.O., Slafer, G.A., 2012. Environmental modulation of yield components in cereals: heritabilities reveal a hierarchy of phenotypic plasticities. Field Crop Res. 127, 215–224.

Shackley, B.J., 2000. Crop Management. Western Australian Department of Agriculture, Perth.

Siddique, K., Belford, R., Perry, M., Tennant, D., 1989. Growth, development and light interception of old and modern wheat cultivars in a Mediterranean-type environment. Aust. J. Agric. Res. 40, 473–487.

Slafer, G.A., 1996. Differences in phasic development rate amongst wheat cultivars independent of responses to photoperiod and vernalisation. A viewpoint of the intrinsic earliness hypothesis. J. Agric. Sci. 126, 403–419.

Slafer, G.A., Rawson, H.M., 1994. Sensitivity of wheat phasic development to major environmental factors: a re-examination of some assumptions made by physiologists and models. Aust. J. Plant Physiol. 21, 393–426.

Slafer, G.A., Rawson, H.M., 1995. Base and optimum temperatures vary with genotype and stage of development in wheat. Plant Cell Environ. 18, 671–679.

Slafer, G.A., Araus, J.L., Royo, C., Del Moral, L.F.G., 2005. Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Aust. J. Agric. Res. 146, 61–70.

Slafer, G.A., Kantolic, A.G., Appendino, M.L., Miralles, D.J., Savin, R., 2009. Crop development: genetic control, environmental modulation and relevance for genetic improvement of crop yield. Crop Physiology. Academic Press, San Diego, pp. 277–308 (Chapter 12).

Slafer, G.A., Savin, R., Sadras, V.O., 2014. Coarse and fine regulation of wheat yield components in response to genotype and environment. Field Crop Res. 157, 71–83.

Sofield, I., Evans, L., Cook, M., Wardlaw, I., 1977. Factors influencing the rate and duration of grain filling in wheat. Funct. Plant Biol. 4, 785–797.

Sreenivasulu, N., Schnurbusch, T., 2012. A genetic playground for enhancing grain number in cereals. Trends Plant Sci. 17, 91–101.

Tondelli, A., Francia, E., Visioni, A., Comadran, J., Mastrangelo, A.M., Akar, T., Al-Yassin, A., Ceccarelli, S., Grando, S., Benbelkacem, A., van Eeuwijk, F.A., Thomas, W.T.B., Stanca, A.M., Romagosa, I., Pecchioni, N., 2014. QTLs for barley yield adaptation to Mediterranean environments in the 'Nure' × Tremoisbiparental population. Euphytica 197, 73–86.

Trevaskis, B., Bagnall, D.J., Ellis, M.H., Peacock, W.J., Dennis, E.S., 2003. MADS box genes control vernalisation-induced flowering in cereals. Proc. Natl. Acad. Sci. USA 100, 13099–13104.

Trevaskis, B., Hemming, M.N., Dennis, E.S., Peacock, W.J., 2007. The molecular basis of vernalisation-induced flowering in cereals. Trends Plant Sci. 12, 352–357.

Turner, A., Beales, J., Faure, S., Dunford, R.P., Laurie, D.A., 2005. Botany: the pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310, 1031–1034.

Vanoosterom, E.J., Acevedo, E., 1992. Adaptation of barley (*Hordeum vulgare* L) to harsh meditereanean environments. 2. Apical development, leaf and tiller appearance. Euphytica 62, 15–27.

Waddington, S.R., Cartwright, P.M., Wall, P.C., 1983. A quantitative scale of spike initial and pistil development in barley and wheat. Ann. Bot. 51, 119–130.

Whitechurch, E.M., Slafer, G.A., Miralles, D.J., 2007. Variability in the duration of stem elongation in wheat and barley genotypes. J. Agron. Crop Sci. 193, 138–145.

Wiegand, C.L., Cuellar, J.A., 1981. Duration of grain filling and kernel weight of wheat as affected by temparature. Crop Sci. 21, 95–101.

Yan, L., Fu, D., Li, C., Blechl, A., Tranquilli, G., Bonafede, M., Sanchez, A., Valarik, M., Yasuda, S., Dubcovsky, J., 2006. The wheat and barley vernalisation gene VRN3 is an orthologue of FT. Proc. Natl. Acad. Sci. USA 103, 19581–19586.

Young, K.J., Elliott, G.A., 1994. Art evaluation of barley accessions for adaptation to the cereal growing regions of Western Australia, based on time to ear emergence. Aust. J. Agric. Res. 45, 75–92.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Res. 14, 415–421.

Zakhrabekova, S., Gough, S.P., Braumann, I., Muller, A.H., Lundqvist, J., Ahmann, K., Dockter, C., Matyszczak, I., Kurowska, M., Druka, A., Waugh, R., Granerd, A., Stein, N., Steuernagel, B., Lundqvist, U., Hansson, M., 2012. Induced mutations in circadian clock regulator Mat-a facilitated short-season adaptation and range extension in cultivated barley. Proc. Natl. Acad. Sci. USA 109, 4326–4331.

CHAPTER 3: Using a novel PLS approach for envirotyping of barley phenology and adaptation

Statement of Authorship

Title of Paper	Using a novel PLS approach for envirotyping of barley phenology and adaptation
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Written in preparation for submission to field crops research journal

Principal Author

Name of Principal Author (Candidate)	Kenton Porker		
Contribution to the Paper	Designed experiments, conducted phenotypic assessments, developed methodology, conducted analysis and wrote the manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	26/2/2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Daniel Cozzolino		
Contribution to the Paper	Assisted with PLS modelling and methodology development for the modelling component, editing and reviewing manuscript		
Signature		Date	26th February 2018

Name of Co-Author	Stewart Coventry

Contribution to the Paper	Idea development of experiments, data interpretation, editing and reviewing manuscript		
Signature		Date	28TH FEBRUARY 2018

Name of Co-Author	Neil Fettell		
Contribution to the Paper	Assisting in phenotyping, Editing and reviewing manuscript		
Signature		Date	26 February 2018

Name of Co-Author	Jason Eglinton		
Contribution to the Paper	Idea development, editing and reviewing manuscript		
Signature		Date	28th February 2018

Using a novel PLS approach for envirotyping of barley phenology and adaptation

Porker K[1]., Coventry S[1]., Fettell NA[2]., Cozzolino D[3]., Eglinton J[4]

1 School of Agriculture, Food and Wine, Faculty of Sciences, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond SA, Adelaide 5064, Australia

2 University of New England and Central West Farming Systems, Condobolin, NSW, 2877, Australia

3 School of Medical and Applied Sciences, CQIRP (Central Queensland Innovation and Research Precinct), Central Queensland University Australia, Bruce Highway, North Rockhampton, Qld 4701, Australia

4 Sugar Research Australia, Bruce Highway, Gordonvale, Qld 4865, Australia

Abstract:

Improving grain yield and adaptation is achieved by synchronising crop phenology with the environment. Phenology research is complex and encounters analytical challenges in characterising genotype x environment (GxE) interactions. This paper presents a simple approach that helps to explain the environmental drivers of phenology. Photoperiod and temperature are major environmental cues for expression of crop developmental genes, and sensitivity to photoperiod is thought to be the major cause of maturity differences among Australian spring barley varieties. However, temperature and photoperiod show similar seasonal trends and strong autocorrelation makes it difficult to distinguish their relative importance in crop development. Partial Least Squares regression (PLS) was developed to handle large data sets with many correlated explanatory variables and only one dependent variable. Across 35 environments encompassing a wide range of temperature and photoperiod regimes in southern Australia, a PLS model described more than 90% of the phenotypic variation in time to anthesis of three adapted barley cultivars. The PLS outputs defined the critical periods when photoperiod and temperature were most influential, and revealed that temperature effects are of equal or greater importance than photoperiod in determining anthesis date, which is a new finding for genotypes adapted to Australian environments. Insight into the previously elusive differential responses to changes in daily average, maximum, and minimum temperatures will assist in developing flowering models for growers that are more accurate, and assist breeders in the genetic dissection of phenology for target environments.

Introduction:

Understanding GxE interaction is a critical aspect of plant breeding. Improving grain yield and adaptation is achieved by synchronising crop phenology phases with resource availability and favourable climate conditions to maximize crop growth (Richards, 1991). Yield has been significantly improved through direct selection for grain yield in low rainfall Mediterranean environments, such as those located in southern Australia, Consequently this has led to indirect selection for phenology allele combinations that facilitate anthesis during the most desirable period to minimise frost, heat, and water stress. Despite yield improvement, understanding of the interaction of major phenology genes with different environmental controls of phenology is poor. The characterization of environmental factors affecting plant growth and development have recently been defined using the concept of "envirotyping" (Cooper et al., 2014; Xu, 2016). Envirotyping complements genotyping and phenotyping allowing better characterization of genotype (and QTL) × environment interaction. Such site-specific characterization and prediction of plant performance will likely be coupled with phenomics, crop growth modelling, and genome-wide prediction (Cooper et al., 2014). Improved characterisation of GxE for barley phenology will facilitate selection of favourable phenology gene combinations in concert with sowing dates that ensure anthesis occurs during the optimal period. It is thought that the major control of spring barley phenology in Australia is photoperiod sensitivity (Boyd et al., 2003), while importance of the sensitivity to vernalising and non-vernalising temperatures is less understood (Ren et al., 2010). There is limited information describing the photoperiodic and temperature environment types and phenology responses in barley cultivars adapted to Australian conditions. Robust characterisation of the genetic responses to thermal and photoperiod regimes are needed to determine annual and inter-annual variations in crop phenology across environments.

The main phenology phases of barley are the vegetative, early reproductive, late reproductive and grain filling phases (Appleyard et al., 1982). Genes that regulate the plant's response to environmental cues control the timing and duration of these phases. The major phenology genes include the photoperiod (*Ppd*), vernalisation (*Vrn*), and earliness *per se* (*Eps or Eam*) genes (Cockram et al., 2007). Allelic variation exists in these genes, enabling commercial production in winter and summer, in regions outside of barley's Mediterranean centre of origin, which differ in temperature, altitude and latitude to those found in southern Australia (Cockram et al., 2007). Both photoperiod and vernalisation affect the rate of specific phenology phases.

In barley, increased photoperiod shortens the time to heading up to an optimum photoperiod, beyond which the time to heading is constant provided vernalisation requirements have been satisfied. Temperature may affect time from sowing to anthesis in two main ways. Firstly, there may be a vernalisation requirement for exposure to low temperature before floral initiation proceeds. The temperature ranges for vernalisation reported in the literature are from -5 to 16 °C, with a maximum effect generally between 0 and 8 °C (Roberts et al., 1988), and from 3 to 12 °C with an optimum of 7 °C (Trione and Metzger, 1970). Secondly, temperature *per se* affects all phases constitutively from sowing to maturity and can be characterised in thermal time units. Over a wide range of temperatures the rate of progress towards anthesis increases with an increase in temperature to an optimum temperature at which anthesis is most rapid (Bonhomme, 2000). At supra-optimal temperatures, flowering is progressively delayed as temperatures get warmer (Roberts et al., 1988). Researchers have always encountered difficulty when trying to identify the temperature measurements and the types of interaction with daylength that best predict the timing of developmental events (Atkinson and Porter, 1996).

Under field conditions, sowing date and location determine the temperatures and photoperiods under which a crop develops. In Mediterranean environments such as southern Australia, spring barley is typically sown in late autumn or early winter, grown through spring, and harvested in early summer. Temperatures are relatively warm along with photoperiods of up to 12.5 hours around early sowing in April. Temperatures decrease thereafter until late winter, after which they increase during spring towards anthesis. Photoperiod follows a similar pattern decreasing after sowing until the shortest photoperiod in June (around 9.5 hours) then increasing through the period of stem elongation back to 12-13 hours by anthesis in September to October. Delayed sowing in southern Australian environments is typically associated with increased photoperiod and temperature during the stem elongation phase, reducing the duration of the emergence to heading phase (Hay and Elliss, 1998).

Controlled experiments have attempted to dissect phenology in several Australian barley genotypes using above optimum photoperiod (up to 18 h) and constant temperature regimes (Kernich et al., 1996; Miralles and Richards, 2000), while most other factors are held constant or their effects ignored (Karsai et al., 2008). While this may be a valid approach, it could be argued that controlled experiments may not be representative of target growing environments in southern Australia and could infer relatively simple relationships between photoperiod and temperature. (Karsai et al., 2008) demonstrated a delay in anthesis with minor temperature

fluctuations in the order of 2 °C, compared to a constant temperature regime. Spring genotypes were the least affected, and facultative genotypes the most affected. These results highlight the need for rigorous characterization of all environmental cues in flowering time experiments.

Phenology analysis models:

Models are often used to support theoretical research, yield predictions and decision making in agriculture. Photoperiodic and temperature regression models have been used to describe phasic development in field crops for many years (Angus et al., 1981) particularly in wheat (Perry et al., 1987; Loss et al., 1990), and in fact empirical thermal models of flowering time date back to before the 20th century (Wang, 1960). Using a simple linear regression model based on photoperiod and temperature, (Alzueta et al., 2014) predicted barley heading time with an accuracy of +/- four days in cultivars not requiring vernalisation. Other crop simulation models such as APSIM (Manschadi et al., 2006; Holzworth et al., 2014), CERES-Barley (Otter-Nacke et al., 1991) and QBAR (Goyne et al., 1996) are powerful tools for predicting phenology. Nevertheless, these models require variables such as soil type, soil moisture and nitrogen levels as inputs and use mathematical algorithms that describe variations in the rate of development over time in response to temperature and photoperiod.

In quantitative genetics, the mean performance of genotypes has traditionally been used to measure the value of that environment. In the simplest form, the Finlay-Wilkinson Regression approach has been a popular method to describe GxE interactions (Finlay and Wilkinson, 1963). This is limited since only the phenotype is used to describe the environment which masks some GxE effects. Uncertainty about the environmental means is ignored, and there is no clear way of incorporating other environmental indicators, pedigree, or molecular marker information when estimating the intercepts and slopes of the regression lines.

Analysing genotype–phenotype relationships requires more robust crop models than those for other agricultural applications. This is possible using a combination of ecophysiological or phenological modelling and QTL analysis (Yin et al., 2005a; Yin et al., 2005b; Chapman, 2008; Hammer et al., 2010) and more than 90% of the variation in flowering time was accounted for by Yin et al. (2005b). More recently, genome-wide models have helped overcome some of the limitations of classical QTL based approaches, which may ignore the effects of QTL with small effects (Uptmoor et al., 2016). Precise estimation of allelic marker effects in response to environmental regulators is required; however, even in current state-of-the-art models, accounting for complex environmental interactions remains a challenge, and at present, best

results are obtained with relatively simple models comprising few parameters (Uptmoor et al., 2016). The key to improving crop adaptation will be to understand the cumulative effect of the environmental factors from sowing that trigger the complex biological processors that control flowering time. Traditional analysis of phenology often does not consider this dynamic nature of GxE making it difficult to derive any significant biological understanding. Robust envirotyping or improved characterisation of phenological environments according to different variables would be more biologically meaningful (Xu, 2016).

Partial Least Squares Regression

An alternative approach for environmental characterisation is Partial Least Squares regression (PLS), a statistical analysis tool developed to handle large datasets and widely used in chemometrics and hyperspectral remote sensing with many auto-correlated variables. Similar challenges exist when analysing phenology data because the environmental input variables influencing phenology are often highly auto-correlated and not distributed evenly. For example, the photoperiods of two consecutive days are more closely correlated than the photoperiods of any other day in the year and the seasonal changes in photoperiod and temperature are highly correlated. Other problems are likely to arise when analysing phenology because the number of independent data variables exceeds the number of dependent variables (i.e. flowering time), particularly if high-resolution weather records are used. Multiple linear regression could be used to handle complex datasets, however, with a large number of factors this can lead to over-fitted models that fail to predict new data well (Wold et al., 2001). In such cases, there may be only a few underlying or latent factors that account for most of the variation in the response.

Wold (1966) introduced the basic statistics of PLS that first construct latent quantitative factor variables forming an X matrix (similar to principal components) from the independent data (e.g. daily temperatures and photoperiod) and then uses these components in regressing a variable Y. The contribution of each individual variable to the PLS model is then evaluated by the standardized model coefficients, with the outputs indicating the direction and magnitude of the effect. If coefficients are positive and high, there is a strong positive correlation between the respective independent variable and the dependent variable (e.g. between temperature and the timing of a phenological stage) (Luedeling et al., 2013; Guo et al., 2015).

Further advancements have led to optimisation techniques where the number of variables to be included in each latent variable matrix can be chosen empirically based on the strength and

significance of the regression component to facilitate biological interpretation. (Luedeling and Gassner, 2012) proposed that PLS is effective for analysing the effect of climatic variables on the variation of biological phenomena, in a standardized procedure, which has been difficult with other methods used to date. PLS regression analysis was used to identify the chilling and forcing periods of temperate fruit trees in Mediterranean climates, and more recently to determine the effects of warming temperatures in walnuts and apricots (Luedeling and Gassner, 2012; Luedeling et al., 2013; Guo et al., 2015) and olives (Aguilera et al., 2015). PLS was used for guiding experimental research in walnuts by identifying critical periods of the season that were important for the timing of key developmental stages, such as budburst. A similar approach in cereals may help to describe phenological environments and identify key periods during the growing season where thermal and photoperiodic regimes influence crop phenology. More recently PLS is finding application in genomic selection where whole genome markers are used to predict and describe a phenotype (Burstin et al., 2015). The advantage of PLS over other approaches is that it identifies only relevant predictor variables, while other linear models require pre-selection of potential predictor variables prior to regression analysis. There are no current studies where PLS has been applied to understand the environmental influences on cereal phenology.

The objectives of this study are: (i) to characterize crop phenology of Australian barley cultivars in response to thermal and photoperiodic environments to identify key phenological environments, and (ii) to assess the application of PLS for its utility in explaining and identifying the phenological responses. The criteria applied for building the model can be applied to other cultivars, crops, and regions to assist in developing a method to dissect the GxE interaction for complex phenotypic traits.

Materials and Methods

Source of data

Experiments were conducted in South Australia in 2014 and 2015 at the Loxton Research Station, Waite Campus (Urrbrae), Strathalbyn (Charlick Research farm) and Roseworthy Agricultural College (Table 1). Each experiment consisted of three to eight sowing dates in 2014 and 2015 using cultivars and unreleased breeding lines adapted to south-eastern Australia. Only the genotypes Compass, Commander, and Fathom will be discussed as they are the highest yielding varieties in these regions (Porker, 2017).

The experiments were split-plot randomised complete block designs with two to five replicates, sowing dates where randomly distributed as the whole plots and varieties randomly allocated within each sowing date. All sites had a seeding density of 150 seeds/m^2 and plot sizes of 2 m x 0.6 m at Loxton, 3 m x 0.6 m at Waite and 3.8 m x 1.28 m at Strathalbyn and Roseworthy. Fertiliser application and weed and disease control matched conventional district practices, and no nutritional or biotic stresses were observed.

Table 1. Description of the experimental sites, showing latitude, longitude, season, sowing date range and the sowing day of the year for each experiment.

Study Location	Coordinates	Year	Sowing date range	Sowing days of year
Loxton	34°26'21.4"S 40°35'55.3"E	2014	15 Apr - 10 Jun	106, 113, 119, 127, 134, 148, 162
		2015	15 Apr - 15 Jun	106, 119, 126, 140, 146, 153, 160, 127
Urrbrae	34°57'56.6"S 138°38'00.2"E	2014	21 Apr - 1 Jul	112, 125, 132, 147, 162, 183
		2015	19 Apr - 29 Jun	110, 124, 138, 152, 181
Strathalbyn	35°19'19.9"S 138°53'02.5"E	2014	27 Apr - 20 Jun	118, 140, 172
		2015	27 Apr - 21 Jun	118, 145, 173
Roseworthy	34°28'51.1"S 138°40'26.9"E	2015	26 Apr - 3 Jul	117, 144, 185

Phenology data

Phenology data was collected from 2 weeks after sowing until anthesis at 2-14 day intervals depending on location and growth stage. Assessments were more frequent around anthesis. A minimum of three plants per genotype was randomly sampled from each plot and the external development was described using Zadoks growth stages (Zadoks et al., 1974). Three m*ain* stems were dissected to observe the apical meristem (Kirby and Appleyard, 1987a; Kirby and Appleyard, 1987b), and the developmental stage was recorded using the scale of (Waddington et al., 1983). The time of anthesis was defined as when 50% of florets within a main stem spike had flowered. The duration from sowing to anthesis was measured in days and thermal time units (TT; °Cd, growing degree days) using 0 °C as a base temperature (Kirby and Appleyard, 1987b; Holzworth et al., 2014). Days and thermal time to anthesis were determined for each genotype in each environment by fitting a linear or polynomial regression of the Waddington

developmental scores against days and accumulated degree days from sowing using GraphPad Prism version 7.00.

Environmental Data

Daily maximum and minimum air temperature, rainfall, and other meteorological data were recorded hourly with meteorological stations at the Roseworthy and Loxton sites. Waite and Strathalbyn daily temperature, rainfall and other meteorological data were obtained from the patched point dataset described by (Jeffrey et al., 2001). Daylength including civil twilight was calculated using the formulae of Forsythe et al. (1995).

Statistical Analysis

Data Pre-processing

For every environment, the daily minimum and maximum temperatures were averaged to obtain daily mean temperature. A 10-day running mean of daily minimum, maximum, and mean temperatures was used to improve PLS modelling by smoothing out the high day-to-day variability in temperatures which can result in poor autocorrelation as reported by (Luedeling and Gassner, 2012). Temperature data for analysis using this approach was generated for each environment for the period starting ten days before and ending 115 days after sowing. This time period was chosen as it is the median flower time for all experiments and captures the majority of temperatures and photoperiod leading up to flowering in each experiment. Photoperiod variables started from 10 days after sowing to reflect emergence and the phase in which the plant begins to respond to the inductive signals of photoperiod.

Full Partial Least Regression (PLS) Model 1:

For the PLS analysis, the dependent variable of interest was thermal time to anthesis. Separate analyses were used for Compass, Commander, and Fathom. The x latent variables comprised a matrix of 460 environmental variables for each of the 35 flowering time observations. The independent environment variables were; daily photoperiod, smoothed daily mean, and minimum and maximum temperatures.

The PLS analysis was conducted using the Unscrambler software (version 10.3, CAMO, Norway) and the NIPALS algorithm, where the data was standardised based on the mean and standard deviation. The dependent and independent variables were centred and scaled to allow comparison between different variables, with respect to their influence in the model. Optimal PLS models were developed with full cross-validation using leave-one-out cross-validation

which identifies significant variables contributing to the best fit of the model (Martens and Martens, 2000). The weighted regression coefficients were significant at $P<0.05$ and the direction and strength of the effect of each variable in the model were generated. The optimal models reported were identified by the optimum number of terms in the PLS calibration models, as determined by the lowest number of factors giving the minimum value of the predicted residual error sum of squares. The coefficient of determination in calibration and cross-validation (R^2), and the root-mean-square error (RMSE) was calculated for the prediction or validation samples (RMSEV) and the calibration samples (RMSECV) to test the predictive ability of the models developed. The regression coefficient profile was obtained by plotting the model coefficients of the standardised data against the predictor variables.

Simplified PLS Model 2:

The statistical approach to Model 2 was dependent on the outputs from Model 1 that identified the periods and environmental variables significantly influencing the time to anthesis. Significant variables from Model 1 were used to create six simplified mean environmental variables. The six variables were used for a simplified (lower resolution) PLS model using the same method as described in Model 1. The accuracy of each model was compared using a correlation analysis.

Genotypic comparison

Simple statistics and ANOVA of flowering time were conducted using GenStat VSN International, Version 15. Finlay–Wilkinson regression (Finlay and Wilkinson, 1963) was used to assess the stability of varieties across different environments by regressing the time to anthesis of each genotype against the environmental means of all three varieties. From the PLS analysis the strength and direction of the variables of importance (weighted regression coefficients) were used to compare the influence of the environment on the time to flower between genotypes for PLS model 1 and 2. Differences in genotype sensitivity to environmental variables were further tested by comparison of partial regression coefficients using a t-test of difference between the significant standardized partial regression coefficients (β) identified from Model 1 during the significant periods.

Identifying phenological environments

PLS models are similar to principal component analysis which results in scores and loadings that may be visualized in a score-loading plot (biplot). Scores and loadings from the optimised PLS Model 2 analysis were used to group and characterise the environments accounting for

most of the variation in time to anthesis. Score plots were used for interpreting relationships among observation sites, and loadings plots were used to interpret relations among environmental variables within each grouping.

APSIM simulation

Simulations to estimate the anthesis date of Commander across all environments were conducted using APSIM version 7.6 (Holzworth et al., 2014). Soil characterization was obtained from the APSoil database (Dalgliesh et al., 2009) and patched-point meteorological weather data from the SILO database (Jeffrey et al., 2001). Manschadi et al. (2006) present a full description of the approach to modelling barley phenology in APSIM. The daily thermal time in APSIM is calculated as crown temperature, and is adjusted by predetermined genetic and environmental factors in APSIM. Therefore, the simulated days to anthesis were compared with observed days to anthesis in a linear regression and predictive statistics are reported.

Results:

Table 2 shows the mean, range and standard deviation of thermal time and days to anthesis for three genotypes across 35 environments. Further information about anthesis dates can be found in Supplementary Table 1. There was a wide range of days to anthesis (92-183) and thermal time to anthesis (980-1615 °C.d) which suggests a strong influence of genotypes and environmental conditions resulting from sowing date and location. The data set was therefore considered appropriate to test the robustness of PLS models to characterise phenology. A multi-site analysis revealed significant GxE (results not shown) for anthesis date: compared to Compass, Commander flowered 78 °C.d later and Fathom 41 °C.d later (Table 2).

Table 2. Descriptive statistics for thermal time and days to anthesis in Compass, Commander and Fathom, indicating range, median, mean and standard deviation across the 35 environments.

	Thermal time to anthesis			Days to anthesis		
	Compass	Commander	Fathom	Compass	Commander	Fathom
Min – Max	1045-1439	980-1608	1009-1615	93-121	89-131	89-130
Median	1251	1325	1287	108	118	114
Mean	1237	1315	1278	108	115	111
Std Deviation	102.4	165.5	155.5	7.7	11.7	11.34

Using the slope in the Finlay-Wilkinson plot as a measure of environmental responsiveness, Compass was the least responsive variety with a slope of 0.72 compared to 1.10 in Fathom

and 1.18 in Commander for thermal days to anthesis (Figure 2). Parameters and estimates from the regression for days to anthesis can be found in Supplementary Table 2 and Supplementary Figure 1.

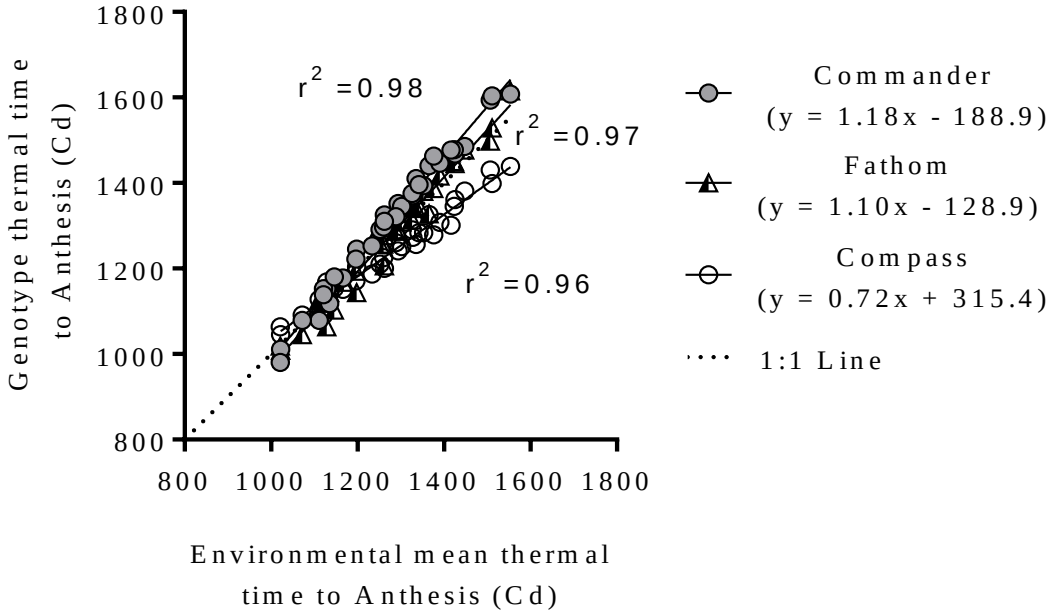


Figure 2. Relationship between the environmental mean of thermal time to anthesis of cultivars, Compass, Commander, and Fathom from 35 growing utilising a combination of site x year x sowing date.

Full PLS Model 1:

Calibration and validation statistics for the observations of thermal time to anthesis in Compass, Commander, and Fathom using the full environmental PLS model 1, and Simplified PLS Model 2 in all 35 environments are shown in Table 3. For thermal time to anthesis the coefficient of determination in cross-validation (R^2) and the RMSECV were 0.92 (RMSECV = 27.9 °C.d) in Compass, 0.95 (RMSECV = 36.5 °C.d in Commander, and 0.89 (RMSECV=52.1 °C.d) in Fathom. For days to anthesis, the R^2 ranged from 0.79 in Compass to 0.92 in Commander, and the RMSECV were 3.3 in Commander and 4.6 in Fathom, respectively. The optimised PLS models developed using the full environmental matrix explained more than 90% of the variability in thermal time to flower in all genotypes. Thermal time to flower will be used in subsequent modelling, as the R^2 of cross-validation was more accurate than predicting days to anthesis; however, the number of days to flower model will be used to compare with APSIM phenology predictions. The calibration and validation statistics for the full environmental PLS model 1 for days to anthesis in all environments can be found

in Supplementary Table 3. The number of PLS factors are derived by the full cross-validation method where the optimum number of terms are determined by the lowest number of factors giving the minimum value of the prediction residual error sum of squares; adding more PLS factors beyond this would not significantly improve the percentage variance explained by the model.

Table 3. Calibration and validation statistics for the observations of thermal time to anthesis in Compass, Commander, and Fathom using the full environmental PLS model 1, and Simplified PLS Model 2 in all 35 environments

	Full PLS Model 1			Simplified PLS Model 2		
	Compass	Commander	Fathom	Compass	Commander	Fathom
R^2 Calibration	0.95	0.96	0.92	0.93	0.95	0.90
R^2 Cross-validation	0.92	0.95	0.90	0.92	0.94	0.87
RMSEC	22.5	29.4	42.9	25.8	33.3	46
RMSECV	27.9	36.5	52.1	26.9	39.3	54
No. of PLS factors	2	2	2	2	2	2

The most significant weighted regression coefficients are determined by the uncertainty test and show the direction and strength of the impact of each variable in the PLS model. Figure 3 shows the optimal loadings derived from the PLS calibration.

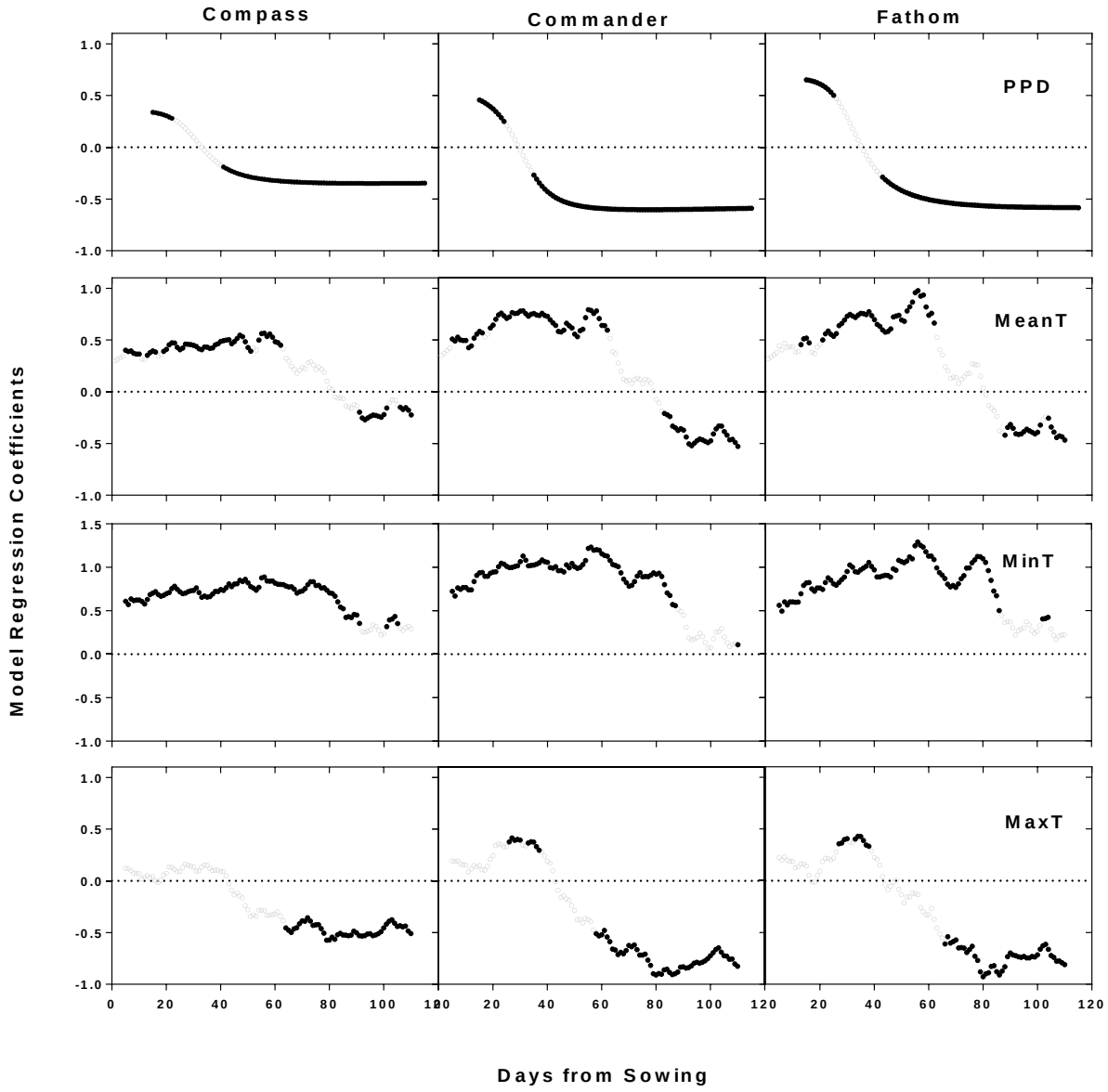


Figure 3. The model weight regression coefficients from PLS model 1 analysis for the growing degree days to anthesis in cultivars (from left to right) Compass, Commander, and Fathom. The four panels from top to bottom display the model coefficients of the centred and scaled data for each cultivar using daily photoperiod (Ppd) and smoothed mean (MeanT), minimum (MinT) and maximum (MaxT) temperatures as independent input variables. The black closed symbols represent the coefficient as a significant variable and the open grey symbols represent a non-significant variation not included in optimised models.

Examination of the loadings (or regression coefficients) is important to identify specific periods and the most important environmental variables related to the thermal time to anthesis. Based on the output demonstrated in Figure 3 seven environmental variables were identified as significantly influencing thermal time to anthesis both negatively and positively at different time periods after sowing and are defined in Table 4

Table 4 Descriptions of the significant latent x environmental variables contributing to the thermal time to anthesis derived from key periods identified in PLS model1

Early_Ppd	Mean daylength (hrs) during the period 15 – 25 days after sowing
Late_Ppd	Mean daylength (hrs) during the period 40 – 115 days after sowing
Early_MeanT	Mean temperature during the period 5 – 60 days after sowing
Late_MeanT	Mean temperature during the period 90 – 110 days after sowing
Early_MinT	Mean minimum temperatures during the period 1-70 after sowing
Early_MaxT*	Mean maximum temperatures for the period 27 – 38 days after sowing
Late_MaxT	Mean maximum temperatures for the period 55- 110 days after sowing

*removed from final simplified model

Genotypic comparisons

The weighted regression coefficients show the direction and strength of the effect of each variable; differences in genotypic sensitivity to the environmental variables can be visualized in the full PLS model for Compass, Commander, and Fathom Figure 3. The response patterns to the environmental variables were similar for all genotypes, although the strengths of the environmental effects differed. The most noticeable difference is the lack of significant effect of maximum temperatures in the period 27 – 38 days after sowing in Compass, compared to a significant effect in Fathom and Commander. Differences in genotype sensitivity to environmental variables were further tested by ANOVA on the significant regression coefficients (β) identified from the full PLS Model 1 during the seven critical periods and corrected for multiple comparisons using a Tukey test (Table 5). Compass always had significantly smaller mean regression coefficient values than Commander and Fathom, suggesting it was less influenced by environmental stimuli (Figure 4). Compass behaved differently to Fathom across all environmental variables whereas Commander and Fathom only differed in responses to Early Ppd, Late_Ppd, and Early MinT and Late_MaxT. Compass and Commander differed in response to all variables apart from Early_Ppd. According to the regression coefficients, in all varieties early minimum temperatures had the largest positive influence on the thermal time to anthesis in the model whereas late maximum temperature had the highest negative influence. Early photoperiods had the largest influence on Fathom, while Commander was more sensitive to late photoperiod, early minimum and late maximum

temperatures than Fathom and Compass, and Compass was the least sensitive to all seven environmental variables.

Table 5. Summary of Tukey multiple comparisons test between Compass, Commander, Fathom for mean regression coefficients over seven environmental sensitive variables and time periods., not significant (ns), **<0.01, ***<0.001, ****<0.0001

	Early_ Ppd	Late_ Ppd	Early_ MeanT	Late_ MeanT	Early_ MinT	Early_ MaxT	Late_ MaxT
No of β values	10	75	55	20	70	11	55
Compass vs. Commander	ns	****	****	****	****	****	****
Compass vs. Fathom	****	****	****	****	****	****	****
Commander vs. Fathom	****	***	ns	ns	****	ns	**

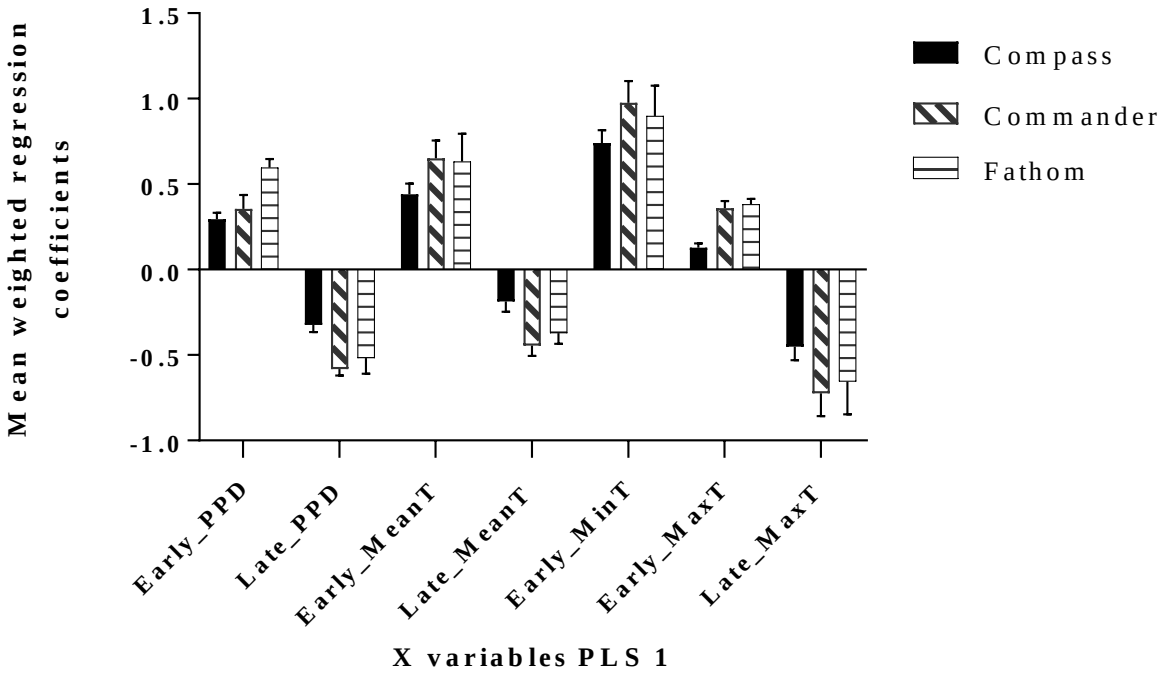


Figure 4. The mean weighted regression coefficients (variables of importance) from the PLS model 1 for thermal time to flower in cultivars Compass, Commander, and Fathom using the x latent variables Early_Ppd, Late_Ppd, Early_MeanT, Late_MeanT, Early_MinT, Early MaxT and Late_MaxT across 35 growing environments, error bars indicate the standard deviation of the weighted regression coefficients.

Simplified PLS Model 2:

The simplified PLS model 2 utilised only the mean environmental variables derived from the seven significant periods identified in Table 4, resulting in a low-resolution matrix of environmental information but capturing the most critical periods of the year controlling the thermal time to anthesis.

Calibration and validation statistics using the simplified environmental PLS model are presented in Table 3 for the thermal time to anthesis in Compass, Commander, and Fathom. Despite an effect in the full environmental model 1, the Early_MaxT did not significantly influence thermal time to anthesis in any genotype in the simplified model so was removed from the final model. The coefficient of determination in cross-validation (R^2) and the RMSECV were 0.92 (RMSECV = 26.9 °C.d) v in Compass, 0.94 (RMSECV = 39.3 °C.d) in Commander, and 0.87 (RMSECV=54 °C.d) in Fathom

Comparison of Models:

Similar trends were observed when the variables of importance (weighted regression coefficients) were compared with the simplified PLS model 2 (Supplementary Figure 2). The R^2 and RMSECV of cross calibration in the simplified model are similar to that of the full environmental model (Table 3) in all genotypes. Importantly, more than 90% of the variability in thermal time to anthesis in all genotypes can be explained by each PLS model. This suggests PLS has excellent application for characterisation of phenology in these environments and the simplified PLS model is an appropriate method to be utilised for easier to interpret environmental characterisation instead of the full environmental matrix used in PLS model 1.

Environmental Characterisation

Scores and loadings plots for Commander (from the simplified PLS Model 2 analysis only) were used to group and characterise the key phenological environments to help describe phenology responses. Commander was selected to characterise the environments because it is the current benchmark genotype for adaptation to Australian environments and represented the greatest response to all environmental variables in this dataset. The scores plot revealed relationships among observational sites and identified six key environment groupings in the first two factors, named ENV1 - 6. The loading plot reveals relationships among environmental variables within each grouping (Figure 5).

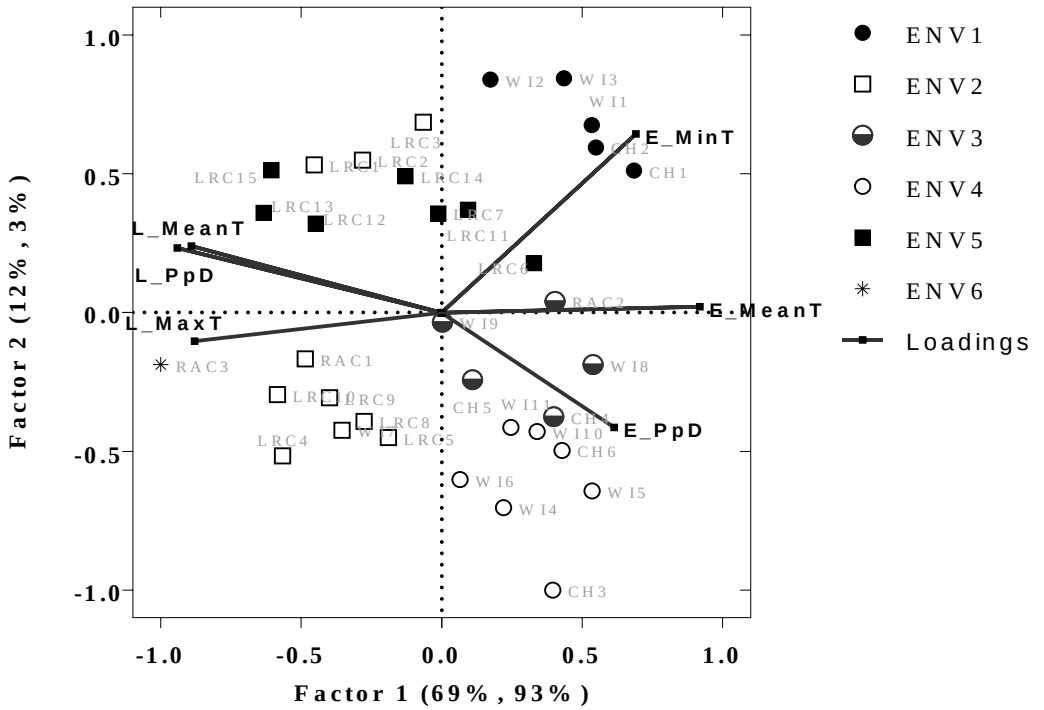


Figure 5 PLS scores and loading plot derived from the simplified PLS model 2 of Commander Barley for the first two factors used to identify key environments to discriminate phenology based on phenotypic and environmental data. Loadings show the x variables contributing to the PLS phenological environments in Factor 1 and 2.

PLS shows clear patterns in the antagonistic effects of early and late temperatures, and early and late photoperiod in factor 1. For example, sites within environment 1 recorded 4° C higher mean and minimum early temperatures compared to environments 5 and 6. The key environmental patterns for each group as defined by the PLS model are summarised in the boxplot diagram (Figure 6) and description in Table 6.

.

Table 6. Description of phenological environment types identified in the PLS analysis.

Environment Type	Description of environment
Environment 1 (ENV1):	Higher mean and minimum temperatures early in the growing season linked to early sowing at Waite and Charlick where conditions are typically milder.
Environment 2 (ENV2):	Higher mean photoperiod and mean temperatures in the early part of the growing season and lower mean photoperiod in the later part of the season corresponding to pre-May planting dates at Loxton and Roseworthy.
Environment 3 (ENV3):	An intermediate environment with few extreme values and comprises approximately the median range for each environmental variable; these sites include typical May planting dates at Charlick, Waite, and Roseworthy
Environment 4 (ENV4):	Higher photoperiods combined with higher mean temperature and higher maximum temperature during the latter part of the growing season corresponding to post May-20 planting dates at Waite and Charlick
Environment 5 (ENV5):	Dominated by below average mean and minimum temperatures during the early part of the growing season and above average photoperiod and maximum temperatures in the late part of the season corresponding to later sowing dates at Loxton in both seasons
Environment 6 (ENV6):	One site sown on the 1st July at Roseworthy characterised by above average late maximum, mean temperature and photoperiod.

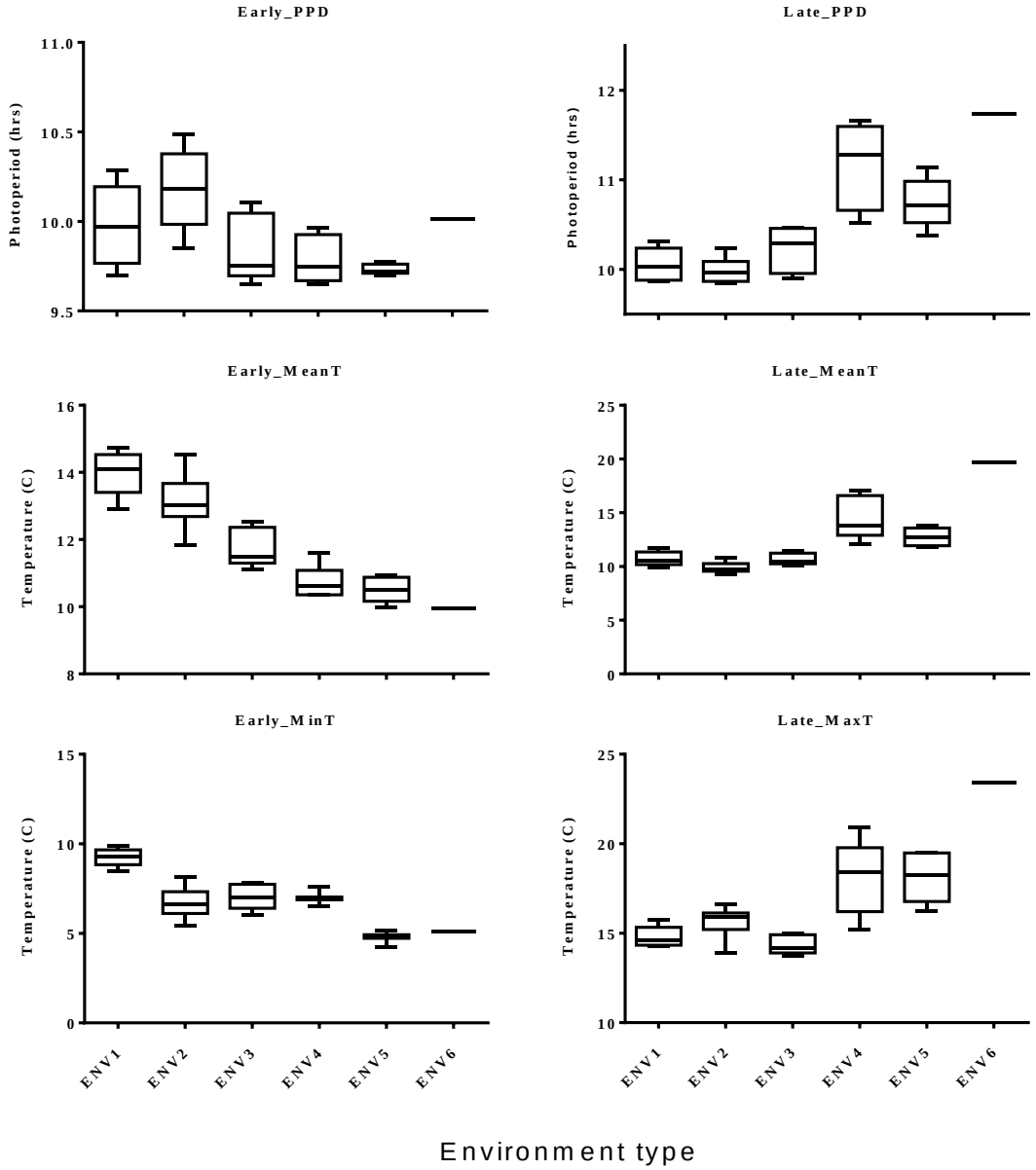


Figure 6 Box plots representative of photoperiod (hrs) for defined variables; Early_Ppd, Late_Ppd and temperature (C) for variables defined previously Early_MeanT, Late_MeanT, Early_MinT, and Late_MaxT in the different environmental types; the boundaries of the box indicate the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles. The horizontal line in the box corresponds to the median value and x indicates the mean.

Genotype by Environment responses

On average across all genotypes, anthesis was delayed in environment 1 to 6 in descending order. However, within and between environments, there were significant changes in the genotypic rankings. In environments 1 and 2, Compass was 150 °C.d earlier to anthesis than Commander and 100 °C.d earlier than Fathom. In the intermediate environment group 3, Compass flowered 100 °C.d earlier than both Commander and Fathom, which were similar.

There was no significant difference between genotypes in environment groups 4 and 5, although Compass flowered 37 °C.d earlier, Commander 115 °C.d earlier and Fathom 80 °C.d earlier than their respective means. Fathom and Commander flowered significantly earlier in environment 6 but not Compass; in this instance, Commander flowered similar to Fathom but 70 °C.d earlier than Compass (Figure 7).

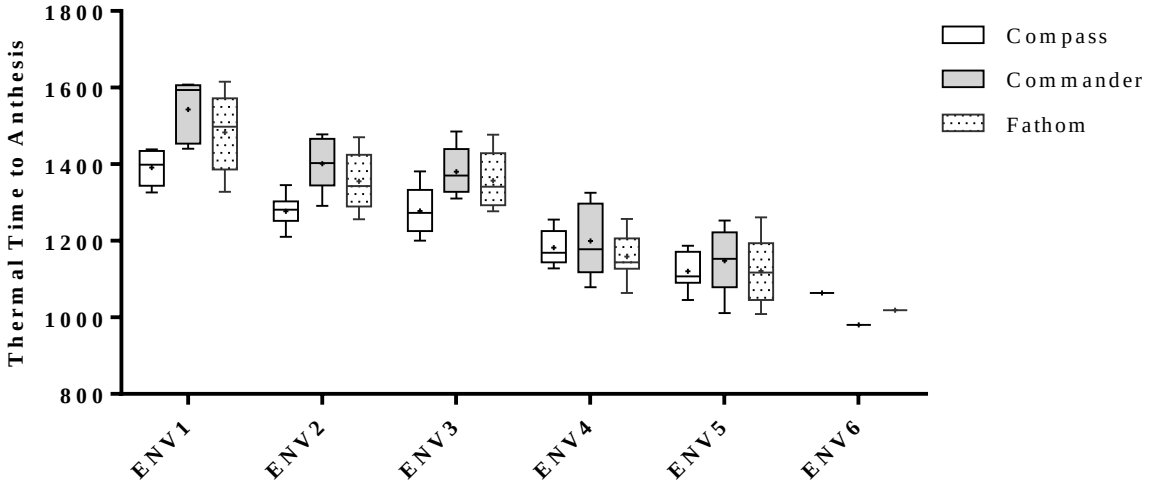


Figure 7. Box plots representative of the thermal time to anthesis for cultivars Compass, Commander, and Fathom in the different environmental types; the boundaries of the box indicate the 25th and 75th percentiles; whiskers indicate the 90th and 10th percentiles. The horizontal line in the box corresponds to the median value and + indicates the mean.

APSIM Comparison

A comparison of APSIM simulated anthesis dates to the observed dates for Commander provides a relatively robust prediction when anthesis occurred after September 5 (day of year 250). However, APSIM predicted earlier flowering dates in situations where flowering occurred before this date by between 2 and 35 days earlier than the observed anthesis day of year. (Figure 8). This also corresponds to earlier planting dates and environments 1 – 3 which are defined by higher mean and minimum temperatures early in the growing season. This suggests the current APSIM model for Commander may not be accounting for the temperature influences observed in the experimental dataset.

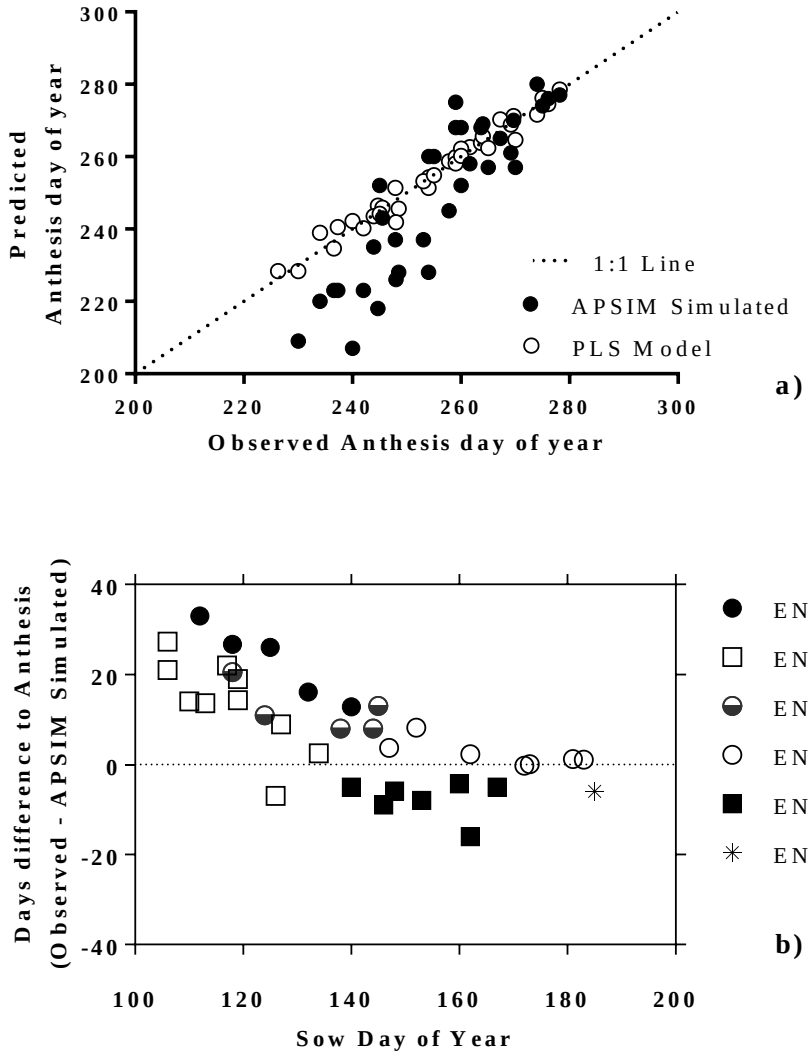


Figure 8a. Relationship between the observed anthesis day of the year and the predicted anthesis day of the year for Commander barley in 35 growing environments using the APSIM model and fitted PLS model, dotted line indicates the 1:1 relationship. b) Relationship between the differences in observed anthesis days and predicted by APSIM compared to the sowing day of the year (from January 1) in each environment group type.

Discussion:

This study has demonstrated that PLS is a novel and robust method of handling complex cereal phenology and climatic data. The approach developed has provided a methodology to identify the most relevant environmental variables that regulate crop development and helped to define phenology envirotypes in southern Australia. Outcomes from this research provide new insight

into GxE interactions, and the temperature and photoperiodic responses that contribute to the adaptation of high yielding barley lines across commercially relevant sowing times.

PLS phenology model

In phenology studies, information on the response of genotypes to the environment is critical for breeders in developing new cultivars and for growers to match planting time with a variety's maturity to achieve an appropriate anthesis date. Crop developmental research encounters analytical challenges in characterising the GxE interactions, largely due to the fact there are many highly auto correlated climate variables, such as photoperiod and temperature that act as cues for crop developmental genes. Using PLS it was possible to fit a complex and simplified model that described more than 90% of the variation in thermal time to anthesis in the three elite genotypes, Compass, Commander, and Fathom, in 35 environments with an accuracy of between 22 and 47 °C.d, which equated to 3 – 4 days, similar to the model used in (Alzueta et al., 2014). This confirms PLS and the methodology used in this study has application for robust characterisation of phenology in these environments, and is in agreement with other studies utilising PLS for phenology for example chilling periods in walnuts (Luedeling and Gassner, 2012) and phenology in apricots (Guo et al., 2015). While the GxE responses can be quantified using traditional approaches such as Finlay and Wilkinson (Finlay and Wilkinson, 1963), measures of trait plasticity (Sadras et al., 2009; Sadras and Slafer, 2012) and linear models (Perry et al., 1987) , they rarely provide insight into the environmental factors that interact with the genotype beyond univariate observations.

Towards improved biological understanding:

Establishing a meaningful relationship between environmental variables and crop development has been the objective of many studies. Statistical modelling approaches such as PLS allow for greater biological understanding and can inform experimental research by first pinpointing the key environmental variables of interest (Luedeling and Gassner, 2012; Luedeling et al., 2013), test hypotheses, and provide new insight into biological processes. PLS identifies only relevant predictor variables, while other linear models require pre-selection of potential predictor variables prior regression analysis.

Using the outputs and interpretation of the significant regression coefficients from the full optimised PLS, it was possible to define critical periods in the plant's life cycle (days after sowing) where the effects of photoperiod and temperature were most influential outlined in Table 4. The six key environmental variables could then be utilised in the simplified lower

resolution model and it was possible to identify six key factorial eco-phenological environments for easier interpretation based on their climatic patterns (Table 6). Simplified models may be preferred in integrating phenotype and genetics in breeding programs, particularly as (Hammer et al., 2006) described successful models generally utilised a coarse level of granularity to capture system dynamics and much of the fine detail is not required.

There was significant GxE interaction for the thermal time to anthesis. The broadly adapted, higher yielding line Compass (Porker, 2017) had the shortest mean duration to anthesis but was also the most stable phenotype in this study recording the smallest variation in thermal time to anthesis across a wide range of environments. Sadras and Richards (2014) discussed possibilities of using phenotypic plasticity for breeding programs rather than direct selection for yield related traits. The stable phenotype of Compass suggests it has lower plasticity than Commander has, due to its lower sensitivity to environmental factors. Therefore, an increased focus on the reduced plasticity of anthesis time may be an important adaptive trait for Australian environments, in addition to a relatively short duration to anthesis. This is particularly relevant given there may be little scope for improving barley adaptability and yield by further adjustments in total time to anthesis and where GxE interaction is large (Boyd et al., 2003; Slafer et al., 2005). However, it must be noted anthesis date of the year rather than plasticity of total time to anthesis should also be considered in the context of the farming system. Particular as varieties that have low sensitivity to environmental crop development controls may flower very early from earlier sowing times and be exposed to frost damage.

The regions explored in this study represent important growing environments for barley in south-eastern Australia. The experiments were grown to determine the developmental response of high yielding barley lines to photoperiod and temperature regimes using different sowing dates and locations. The phenology drivers of well-adapted barley cultivars are poorly described in the literature, it has long been recognised the best-adapted barley cultivars for Australian low-medium rainfall environments are early-maturing spring types, which exhibit a relatively high photoperiod response to achieve a short duration to heading (Boyd et al., 2003; Read et al., 2003). However, in a new finding for Australian environments, these results using PLS analysis show that, under field conditions, Compass was less responsive to photoperiod and has a shorter time to anthesis than the current benchmark Commander and Fathom. This suggests other genetic and environmental factors that previously have not been considered may be equally or more important in regulating anthesis time.

The influence of other environmental factors is highlighted by the fact that thermal time to anthesis varied among genotypes and between environments of similar planting and photoperiod. The differences in development between these environments could be explained by the temperatures experienced in the period between sowing and anthesis. Higher minimum and mean temperatures during the first seventy days delayed anthesis by a maximum of 200 °C.d, which suggests there is a significant effect of minimum temperatures having a vernalisation-like response. This is noticeable in environment types 2 – 6 (Figure 6), where early minimum temperatures are within the range for maximum vernalisation effect between 0 and 8 °C (Roberts et al., 1988) whereas environment one was warmer and resulted in longer times to anthesis. This is an important finding given that all previous literature describing most adapted Australian cultivars and spring types introduced from Europe, Canada, and Japan have either no or a very minimal vernalisation requirement. While there are some exceptions with the cultivar's Ulandra, Urambie and Yambla; these cultivars are not widely grown but maybe useful for very early sowing (Boyd et al., 2003) .

The other period sensitive to temperature occurred just prior to anthesis, where high maximum temperatures reduced the thermal time to anthesis. This was particularly pronounced in Commander grown at Roseworthy in 2015 and environment six where delayed sowing resulting in the crop being exposed to significantly higher temperatures during this period. These findings provide further evidence that differences in anthesis time among varieties exist for reasons other than photoperiod. This should not come as a surprise, however, quantifying the responses to temperature in the field have remained relatively elusive. Ren et al. (2010) noted large differences in heading dates among genotypes between Australian and Southeast Asian environments, despite similar latitudes. These studies indicated there were genes conditioned by temperature differences between the locations, and suggested that variation in duration to heading existed for reasons other than vernalisation and photoperiod. This may be an important adaptive trait for Australian cultivars. Temperature effects could be equal to or even greater than some of the most extreme responses recorded for vernalisation or photoperiod (Read et al., 2003) when grown under milder winter growing conditions. However, it must be noted that all varieties were responsive to photoperiod and it remains an important trait for Australian environments.

In the context of phenology, PLS provides new insight into the previously elusive differential responses to the subtle changes in average, maximum and minimum temperatures and provides evidence for the need to include differential effects of temperature during different seasons into

explanatory models of the effects of temperature on phenology. APSIM-Barley has proven to be robust in simulating the response of barley crops to management and environmental conditions at experimental sites and in farmers' fields (Manschadi et al., 2006). However, the model's capacity to simulate crop phenology reliably in some environments creates challenges for current APSIM users. Comparison with APSIM drawn in these experiments suggests that the current APSIM model used for Commander may not be accounting for the temperature influences previously discussed particularly at earlier sowing dates.

In the case of other crop models, photoperiods are often extended artificially to determine photoperiod coefficient factors and temperature studies have frequently been studied in controlled growth rooms (Ellis et al., 1988; Kernich et al., 1995; Kernich et al., 1996; Karsai et al., 2008). The generation of model coefficients in this way inherently assumes that the genotypic temperature and photoperiod sensitivity factors are fixed in all environments, whereas in fact there may be genes that differ in their sensitivity depending on the level of environmental stimulus. The use of PLS as an analytical tool has helped to investigate these relationships in barley under field conditions, without the need for systematic manipulation of the growing environment. It allows exploration of a wide range of environmental conditions similar to those experienced by the barley crop in field conditions and at the crop level of organisation.

One of the limitations of PLS for use as a predictive model lies in the fact that it is only valid for the particular conditions under which observed data was obtained. Thus, it could be argued that they are not directly useful for predicting biological processes outside the climatic domain of the observations (i.e. at different locations or for climate change scenarios). Although the experiment to obtain the variables to build the model was carried out over two growing seasons and three sites, the fact that cultivars were sown in a wide range of sowing dates ensured that the different genotypes explored a wide range of environments with different temperatures and photoperiods.

Future applications:

More generally, the PLS method used has application for characterisation of phenology in these environments. This model will now be used as a method to analyse other phenological data collected to assist in the understanding of pre-anthesis development, such as the critical period when the number of grains is determined (Arisnabarreta and Miralles, 2008) or stages such as double ridge when barley transitions from the vegetative to reproductive phase. An extended

approach could help develop criteria to be applied to other cultivars or crops and to other regions. This method could be useful for explaining quantitative variation in biological events or the outcomes of biological processes through analysis of full-season records of temperature, precipitation or other environmental variables. Crop models have previously been used to study impacts of extreme climate, as they allow correction for the effect of multiple environmental factors and allow testing of multiple genotype-environment-management combinations (e.g. sowing date × variety) (Hammer et al., 2006). PLS analysis of environmental parameters could find application in various contexts, e.g. for explaining crop yields or for characterizing the vulnerability of farming systems to climate variability during certain phases of crop growth.

The examination of the differences between genotypic environmental loadings (regression coefficients) provide insights into aspects of crop phenology that can be used for genetic dissection. Phenotype by genotype prediction based on eco-physiological models, which account for allelic gene, QTL, or marker effects, have many possible applications in plant breeding programs. (Uptmoor et al., 2016) suggested that, in order for such models to become more applicable, a precise estimation of allelic marker effects in response to environmental regulators is required for improving models predicting phenotype by genotype. Using PLS crop models in the genomic prediction of heading date may be of practical importance if there is a large variation in heading date in target environments of commercial cultivars. The next obvious step is to combine a PLS approach using environmental parameters identification outlined in this research with genomic data using a wider range of cultivars to determine the functional effects of key crop developmental genes on crop phenology. Hammer et al. (2006) suggested novel modelling approaches are needed to predict gene-to-phenotype associations, and to assist with the complexity and scales of biological organization for breeding improved crop plants. There are a number of alternative pathways in the literature, which will all enable an increased understanding of gene-to-phenotype systems for complex traits.

Conclusion:

Using the framework of phenology as a proof of concept for a complex trait, this study has shown that PLS is a robust method to extract meaningful biological explanations from large data sets. It was possible to define critical periods where the effects of photoperiod and temperature were most influential in Australian barley cultivars. This has helped quantify the effect of subtle changes in temperature on barley, providing new evidence that the effect of

temperature may be of equal or greater importance than photoperiod in determining the total thermal time to anthesis. Not only should these effects be considered in future crop models but integrated with genomic data to investigate aspects of crop phenology that can be used for genetic dissection and the design of new ideotypes adapted to Australian environments. PLS has proven its usefulness for envirotyping and paves the way for development of a four-dimensional profile of crop science involving genotype (*G*), phenotype (*P*), envirotype (*E*) and time (*T*) (developmental stage) as proposed by (Xu, 2016).

Acknowledgments: Members of the Adelaide University barley research group for field trial management, and Michael Laws for collection of plants.

Funding: The Grains Research and Development Corporation, the Playford Trust, and the University of Adelaide supported this work.

References:

Aguilera, F., Fornaciari, M., Ruiz-Valenzuela, L., Galán, C., Msallem, M., Dhiab, A.B., la Guardia, C.D.-d., del Mar Trigo, M., Bonofiglio, T., Orlandi, F., 2015. Phenological models to predict the main flowering phases of olive (Olea europaea L.) along a latitudinal and longitudinal gradient across the Mediterranean region. International Journal of Biometeorology 59, 629-641.

Alzueta, I., Arisnabarreta, S., Abeledo, L.G., Miralles, D.J., 2014. A simple model to predict phenology in malting barley based on cultivar thermo-photoperiodic response. Computers and Electronics in Agriculture 107, 8-19.

Angus, J.F., Mackenzie, D.H., Morton, R., Schafer, C.A., 1981. Phasic development in field crops II. Thermal and photoperiodic responses of spring wheat. Field Crops Research 4, 269-283.

Appleyard, M., Kirby, E.J.M., Fellowes, G., 1982. Relationships between the duration of phases in the pre-anthesis life cycle of spring barley. Australian Journal of Agricultural Research 33, 917-925.

Arisnabarreta, S., Miralles, D.J., 2008. Critical period for grain number establishment of near isogenic lines of two- and six-rowed barley. Field Crops Research 107, 196-202.

Atkinson, D., Porter, J.R., 1996. Temperature, plant development and crop yields. Trends in Plant Science 1, 119-124.

Bonhomme, R., 2000. Beware of comparing RUE values calculated from PAR vs solar radiation or absorbed vs intercepted radiation. Field Crops Research 68, 247-252.

Boyd, W.J.R., Li, C.D., Grime, C.R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Kamali, M.R.J., Barr, A.R., Moody, D.B., Lance, R.C.M., Logue, S.J., Raman, H., Readj, B.J., 2003. Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. Australian Journal of Agricultural Research 54, 1277-1301.

Burstin, J., Salloignon, P., Chabert-Martinello, M., Magnin-Robert, J.-B., Siol, M., Jacquin, F., Chauveau, A., Pont, C., Aubert, G., Delaitre, C., Truntzer, C., Duc, G., 2015. Genetic diversity and trait genomic prediction in a pea diversity panel. BMC Genomics 16, 105.

Chapman, S.C., 2008. Use of crop models to understand genotype by environment interactions for drought in real-world and simulated plant breeding trials. Euphytica 161, 195-208.

Cockram, J., Jones, H., Leigh, F.J., O'Sullivan, D., Powell, W., Laurie, D.A., Greenland, A.J., 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. Journal of Experimental Botany 58, 1231-1244.

Cooper, M., Messina, C.D., Podlich, D., Totir, L.R., Baumgarten, A., Hausmann, N.J., Wright, D., Graham, G., 2014. Predicting the future of plant breeding: complementing empirical evaluation with genetic prediction. Crop and Pasture Science 65, 311-336.

Dalgliesh, N.P., Foale, M.A., McCown, R.L., 2009. Re-inventing model-based decision support with Australian dryland farmers. 2. Pragmatic provision of soil information for paddock-specific simulation and farmer decision making. Crop and Pasture Science 60, 1031-1043.

Ellis, R.H., Roberts, E.H., Summerfield, R.J., Cooper, J.P., 1988. Environmental Control of Flowering in Barley (Hordeum vulgare L.). II. Rate of Developement as a Function of Temperature and Photoperiod and its Modification by Low-temperature Vernalization. Annals of Botany 62, 145-158.

Finlay, K., Wilkinson, G., 1963. The analysis of adaptation in a plant-breeding programme. Australian Journal of Agricultural Research 14, 742-754.

Forsythe, W.C., Rykiel, E.J., Stahl, R.S., Wu, H.-i., Schoolfield, R.M., 1995. A model comparison for daylength as a function of latitude and day of year. Ecological Modelling 80, 87-95.

Goyne, P., Meinke, H., Milroy, S., Hammer, G., Hare, J., 1996. Development and use of a barley crop simulation model to evaluate production management strategies in north-eastern Australia. Crop and Pasture Science 47, 997-1015.

Guo, L., Dai, J., Wang, M., Xu, J., Luedeling, E., 2015. Responses of spring phenology in temperate zone trees to climate warming: A case study of apricot flowering in China. Agricultural and Forest Meteorology 201, 1-7.

Hammer, G., Cooper, M., Tardieu, F., Welch, S., Walsh, B., van Eeuwijk, F., Chapman, S., Podlich, D., 2006. Models for navigating biological complexity in breeding improved crop plants. Trends in Plant Science 11, 587-593.

Hammer, G.L., Van Oosterom, E., McLean, G., Chapman, S.C., Broad, I., Harland, P., Muchow, R.C., 2010. Adapting APSIM to model the physiology and genetics of complex adaptive traits in field crops. Journal of Experimental Botany 61, 2185-2202.

Hay, R.K.M., Elliss, R.P., 1998. The Control of Flowering in Wheat and Barley: What Recent Advances in Molecular Genetics Can Reveal. Annals of Botany 82, 541-554.

Holzworth, D.P., Huth, N.I., deVoil, P.G., Zurcher, E.J., Herrmann, N.I., McLean, G., Chenu, K., van Oosterom, E.J., Snow, V., Murphy, C., Moore, A.D., Brown, H., Whish, J.P.M., Verrall, S., Fainges, J., Bell, L.W., Peake, A.S., Poulton, P.L., Hochman, Z., Thorburn, P.J., Gaydon, D.S., Dalgliesh, N.P., Rodriguez, D., Cox, H., Chapman, S., Doherty, A., Teixeira, E., Sharp, J., Cichota, R., Vogeler, I., Li, F.Y., Wang, E., Hammer, G.L., Robertson, M.J., Dimes, J.P., Whitbread, A.M., Hunt, J., van Rees, H.,

McClelland, T., Carberry, P.S., Hargreaves, J.N.G., MacLeod, N., McDonald, C., Harsdorf, J., Wedgwood, S., Keating, B.A., 2014. APSIM - Evolution towards a new generation of agricultural systems simulation. Environmental Modelling and Software 62, 327-350.

Jeffrey, S.J., Carter, J.O., Moodie, K.B., Beswick, A.R., 2001. Using spatial interpolation to construct a comprehensive archive of Australian climate data. Environmental Modelling and Software 16, 309-330.

Karsai, I., Szűcs, P., Kőszegi, B., Hayes, P.M., Casas, A., Bedő, Z., Veisz, O., 2008. Effects of photo and thermo cycles on flowering time in barley: a genetical phenomics approach. Journal of Experimental Botany 59, 2707-2715.

Kernich, G.C., Halloran, G.M., Flood, R.G., 1996. Constant and interchanged photoperiod effects on the rate of development in barley (Hordeum vulgare). Australian Journal of Plant Physiology 23, 489-496.

Kernich, G.C., Slafer, G.A., Halloran, G.M., 1995. Barley development as affected by rate of change of photoperiod. Journal of Agricultural Science 124, 379-388.

Kirby, E., Appleyard, M., 1987a. Cereal development guide. NAC Cereal Unit, Stoneleigh, UK.

Kirby, E.J.M., Appleyard, M., 1987b. Cereal development guide. Arable Unit, National Agricultural Centre, Kenilworth, England.

Loss, S., Perry, M., Anderson, W., 1990. Flowering times of wheats in south-western Australia: a modelling approach. Australian Journal of Agricultural Research 41, 213-223.

Luedeling, E., Gassner, A., 2012. Partial Least Squares Regression for analyzing walnut phenology in California. Agricultural and Forest Meteorology 158–159, 43-52.

Luedeling, E., Kunz, A., Blanke, M.M., 2013. Identification of chilling and heat requirements of cherry trees—a statistical approach. International Journal of Biometeorology 57, 679-689.

Manschadi, A., Hochman, Z., Mclean, G., DeVoil, P., Holzworth, D., Meinke, H., 2006. APSIM-Barley model–Adaptation of a wheat model to simulate barley growth and development. 13th Australian Agronomy Conference: Perth, Western Australia.

Martens, H., Martens, M., 2000. Modified Jack-knife estimation of parameter uncertainty in bilinear modelling by partial least squares regression (PLSR). Food quality and preference 11, 5-16.

Miralles, D.J., Richards, R.A., 2000. Responses of leaf and tiller emergence and primordium initiation in wheat and barley to interchanged photoperiod. Annals of Botany 85, 655-663.

Otter-Nacke, S., Ritchie, J., Godwin, D., Singh, U., 1991. A Users Guide to CERES Barley v. 2.10. International Fertilizer Development Center, Muscle Shoals, AL.

Perry, M., Siddique, K., Wallace, J., 1987. Predicting phenological development for Australian wheats. Australian Journal of Agricultural Research 38, 809-819.

Porker, K., 2017. Barley variety sowing guide 2017. SARDI 2017 Sowing Guide.

Read, B.J., Raman, H., McMichael, G., Chalmers, K.J., Ablett, G.A., Platz, G.J., Raman, R., Genger, R.K., Boyd, W.J.R., Li, C.D., Grime, C.R., Park, R.F., Wallwork, H., Prangnell, R., Lance, R.C.M., 2003. Mapping and QTL analysis of the barley population Sloop × Halcyon. Australian Journal of Agricultural Research 54, 1145-1153.

Ren, X.F., Li, C.D., Boyd, W.J.R., Westcott, S., Grime, C.R., Sun, D.F., Lance, R., 2010. QTLs and their interaction determining different heading dates of barley in Australia and China. Crop & Pasture Science 61, 145-152.

Richards, R.A., 1991. Crop improvement for temperate australia - future opportunities. Field Crops Research 26, 141-169.

Roberts, E.H., Summerfield, R.J., Cooper, J.P., Ellis, R.H., 1988. Environmental-control of flowering in barley (hordeum-vulgare-l) .1. Photoperiod limits to long-day responses, photoperiod-insensitive phases and effects of low-temperature and short-day vernalization. Annals of Botany 62, 127-144.

Sadras, V., Reynolds, M., De la Vega, A., Petrie, P., Robinson, R., 2009. Phenotypic plasticity of yield and phenology in wheat, sunflower and grapevine. Field Crops Research 110, 242-250.

Sadras, V.O., Richards, R.A., 2014. Improvement of crop yield in dry environments: benchmarks, levels of organisation and the role of nitrogen. Journal of Experimental Botany 65, 1981-1995.

Sadras, V.O., Slafer, G.A., 2012. Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. Field Crops Research 127, 215-224.

Slafer, G.A., Araus, J.L., Royo, C., Del Moral, L.F.G., 2005. Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. Annals of Applied Biology 146, 61-70.

Trione, E.J., Metzger, R.J., 1970. Wheat and Barley Vernalization in a Precise Temperature Gradient1. Crop Science 10, 390-392.

Uptmoor, R., Pillen, K., Matschegewski, C., 2016. Combining genome-wide prediction and a phenology model to simulate heading date in spring barley. Field Crops Research.

Waddington, S.R., Cartwright, P.M., Wall, P.C., 1983. A quantitative scale of spike initial and pistil development in barley and wheat. Annals of Botany 51, 119-130.

Wang, J.Y., 1960. A Critique of the Heat Unit Approach to Plant Response Studies. Ecology 41, 785-790.

Wold, H., 1966. Estimation of principal components and related models by iterative least squares. Multivariate Analysis. Edited by: Krishnaiaah PR. 1966. New York: Academic Press.

Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemometrics and Intelligent Laboratory Systems 58, 109-130.

Xu, Y., 2016. Envirotyping for deciphering environmental impacts on crop plants. TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik 129, 653-673.

Yin, X., Struik, P.C., Tang, J., Qi, C., Liu, T., 2005a. Model analysis of flowering phenology in recombinant inbred lines of barley. Journal of Experimental Botany 56, 959-965.

Yin, X., Struik, P.C., van Eeuwijk, F.A., Stam, P., Tang, J., 2005b. QTL analysis and QTL-based prediction of flowering phenology in recombinant inbred lines of barley. Journal of Experimental Botany 56, 967-976.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Research 14, 415-421.

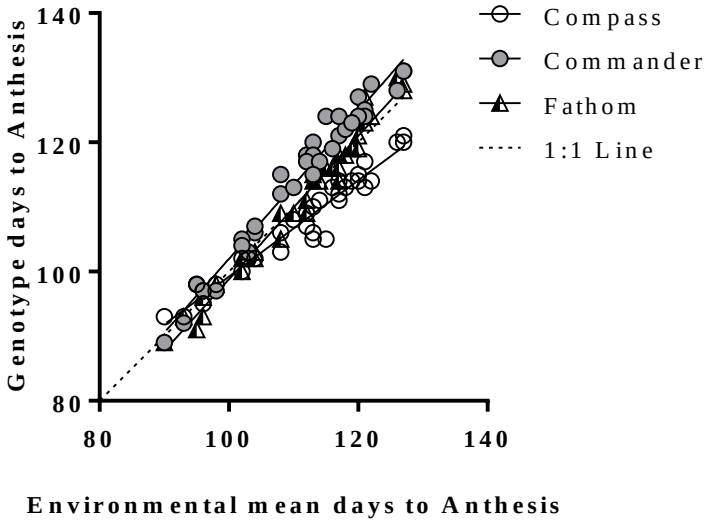
Supplementary Tables & Figures

Supplementary Table 1 Individual site information, including PLS environment group, sowing date, days to anthesis after sowing, thermal time to anthesis after sowing for Compass, Commander and Fathom

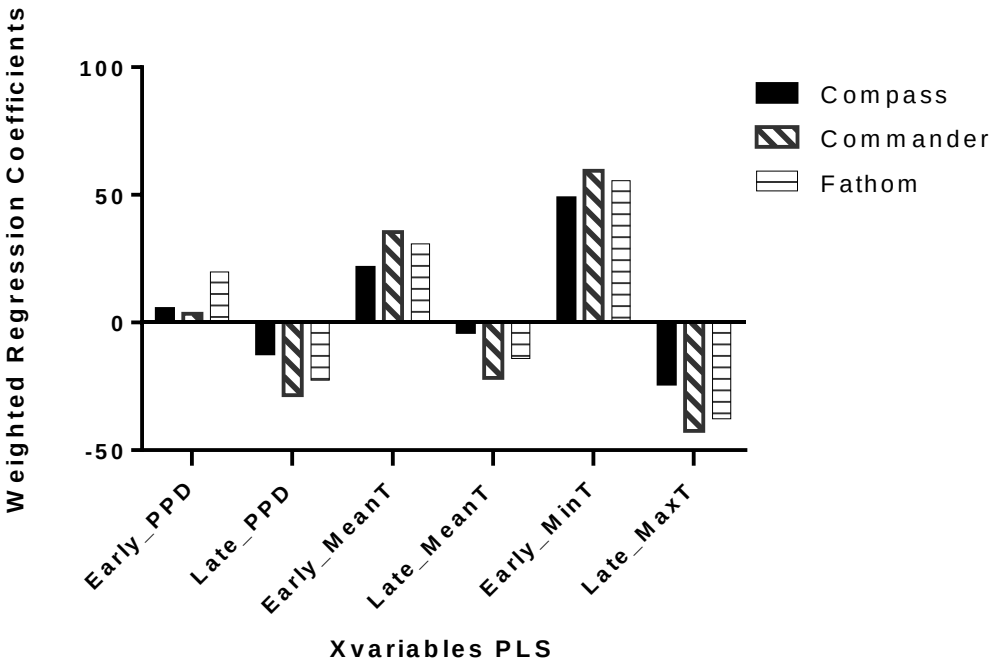
PLS Environment Group	Site	Sowing Date	Year	Sowing day of year	Days to Anthesis after sowing			Thermal time to Anthesis after sowing		
					Compass	Commander	Fathom	Compass	Commander	Fathom
ENV1	Strathalbyn	27-Apr	2014	118	114	127	119	1430	1594	1498
	Strathalbyn	19-May	2014	140	109	118	109	1326	1440	1328
	Waite	21-Apr	2014	112	120	128	130	1439	1608	1615
	Waite	4-May	2014	125	114	129	124	1399	1604	1529
	Waite	11-May	2014	132	111	121	118	1361	1468	1445
ENV2	Loxton	15-Apr	2014	106	105	120	115	1308	1446	1416
	Loxton	22-Apr	2014	113	105	124	116	1279	1463	1387
	Loxton	28-Apr	2014	119	106	118	114	1256	1410	1340
	Loxton	6-May	2014	127	107	117	111	1241	1352	1290
	Loxton	13-May	2014	134	103	112	109	1210	1291	1256
	Waite	19-Apr	2015	110	113	124	127	1345	1478	1450
	Loxton	15-Apr	2015	106	112	124	114	1290	1375	1314
	Loxton	28-Apr	2015	119	114	123	119	1284	1396	1345
	Loxton	5-May	2015	126	113	119	116	1260	1321	1287
	Roseworthy	26-Apr	2015	117	121	131	128	1301	1477	1470
ENV3	Strathalbyn	27-Apr	2015	118	120	131	129	1381	1485	1477
	Strathalbyn	24-May	2015	145	117	125	123	1284	1394	1380
	Waite	3-May	2015	124	115	124	121	1273	1370	1341
	Waite	17-May	2015	138	113	122	118	1251	1345	1309
	Roseworthy	23-May	2015	144	114	121	116	1200	1310	1277
ENV4	Strathalbyn	20-Jun	2014	172	98	98	91	1169	1153	1064
	Waite	26-May	2014	147	106	115	105	1255	1325	1206
	Waite	10-Jun	2014	162	102	105	100	1204	1245	1144
	Waite	1-Jul	2014	183	93	92	93	1128	1079	1127
	Strathalbyn	21-Jun	2015	173	102	103	103	1151	1178	1168
	Waite	31-May	2015	152	111	117	114	1225	1297	1257
	Waite	29-Jun	2015	181	98	97	98	1144	1118	1144
ENV5	Loxton	27-May	2014	148	102	106	103	1090	1153	1119
	Loxton	10-Jun	2014	162	97	97	93	1045	1011	1009
	Loxton	19-May	2015	140	110	115	115	1187	1253	1261
	Loxton	25-May	2015	146	108	113	109	1171	1222	1194
	Loxton	1-Jun	2015	153	102	107	102	1154	1180	1103
	Loxton	8-Jun	2015	160	100	104	102	1107	1139	1117
	Loxton	15-Jun	2015	167	95	97	96	1091	1079	1045
ENV6	Roseworthy	3-Jul	2015	185	93	89	89	1064	980	1019

Supplementary Table 2. Parameters of the regression between the environmental mean time to anthesis in thermal time and days, in Compass, Commander, and Fathom for 35 growing environments. In all case the relationships were significant ($p<0.0001$)

	Thermal time (C.d)			Days		
Genotype	Intercept ± S.E.	Slope ± S.E.	r^2	Intercept ± S.E.	Slope ± S.E.	r^2
Compass	315.4 ± 29.8	0.72 ± 0.023	0.96	24.81 ± 3.36	0.74 ± 0.031	0.94
Commander	-188.9 ± 30.0	1.18 ± 0.023	0.98	-12.29 ± 3.74	1.14 ± 0.033	0.97
Fathom	-128.0 ± 38.6	1.10 ± 0.030	0.97	-12.04 ± 3.33	1.11 ± 0.029	0.97



Supplementary Figure 1. Relationship between the environmental mean of days to anthesis of cultivars, Compass, Commander, and Fathom for 35 growing environments and genotypic days to anthesis.



Supplementary Figure 2. Weighted regression coefficients (variables of importance) from the simplified PLS model 2 for growing degree days anthesis in cultivars Compass, Commander, and Fathom using the x latent variables Early_Ppd, Late_Ppd, Early_MeanT, Late_MeanT, Early_MinT, and Late_MaxT across 35 growing environments. For a description of these abbreviations, refer to Table 5.

Supplementary Table 3. Calibration and validation statistics for the observations of days to anthesis in Compass, Commander, and Fathom using the full environmental PLS model 1 in all 35 environments

	Days to anthesis		
	Compass	Commander	Fathom
R^2 Calibration	0.91	0.95	0.92
R^2 Cross-validation	0.79	0.92	0.84
RMSEC	2.28	2.33	3.14
RMSECV	3.78	3.31	4.58
No. of PLS factors	4	3	3

CHAPTER 4: Developmental variation driving yield and adaptation of barley to Mediterranean environment

Statement of Authorship

Title of Paper	Developmental variation driving yield and adaptation of barley to Mediterranean environments
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Kenton Porker
Contribution to the Paper	Designed and conducted experiments, analysis, and interpreted data, wrote manuscript, edited manuscript and acted as corresponding author.
Overall percentage (%)	75%
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: 26 February 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Glenn McDonald
Contribution to the Paper	Assisted with data interpretation, Editing and reviewing manuscript
Signature	Date: 27/2/18.

Name of Co-Author	Neil Fettell

Contribution to the Paper	Idea development, experimental design and methodology, editing and reviewing manuscript		
Signature		Date	27 February 2018

Name of Co-Author	Jason Eglinton		
Contribution to the Paper	Idea development, editing and reviewing manuscript		
Signature		Date	28th February 2018

Name of Co-Author	Stewart Coventry		
Contribution to the Paper	Idea development, data interpretation editing and reviewing manuscript		
Signature		Date	28TH FEBURARY 2018

Please cut and paste additional co-author panels here as required.

Developmental variation driving yield and adaptation of barley to Mediterranean environments

Porker K[1]., Coventry S[1]., Fettell NA[2]., McDonald GK[1] & Eglinton J[3]

1 School of Agriculture, Food and Wine, Faculty of Sciences, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond SA, Adelaide 5064, Australia

2 University of New England and Central West Farming Systems, Condobolin, NSW, 2877, Australia

3 Sugar Research Australia, Bruce Highway, Gordonvale, Qld 4865, Australia

Abstract

The release of the barley variety 'Compass' represented a substantial improvement in the yield potential of barley adapted to southern Australia, and the reasons for the high yield of Compass are examined using analysis of phenology and yield components. Crop development is the main factor driving yield and adaptation and Compass has a pattern of development that is different to many of the current varieties. Developmental patterns strongly influence grain yield formation, particularly grain number which is primarily determined during the stem elongation period; understanding how development influences yield in Compass provides insight into raising the yield potential of barley in southern Australia. This study describes the variation in developmental patterns and yield of Compass and 11 other elite barley cultivars in six growing conditions across April, May and June planting dates. There was significant variation among genotypes for the duration of the pre-anthesis phases but in most instances these were not independent of time to anthesis and there was no clear link with grain yield. There was little evidence to suggest lengthening the period of stem elongation will improve spikelet survival or grain yield. Through selective breeding, the time to anthesis has been reduced without compromising yield potential and it may be possible to continue to shorten the period of time

from double ridge to awn primordia. Compass has a faster development rate than Commander and its higher yield was associated with modest to intermediate improvements in grain number without any trade-off in grain weight. Due to the dynamic nature of two-row barley and despite variation for phenology, we conclude that a dual focus on direct selection for an appropriate flowering time and yield still remains one of the most effective approaches to optimise development patterns and the dynamics of grain yield.

Introduction

In many malting barley-growing regions around the world, cultivars with superior adaptation, high yield, and grain weight stability, are favoured. Crop development is known to be the main factor driving crop yield and adaptation (Richards, 1991) in Australia, and understanding yield and adaptation within the framework of crop development is of critical importance to breeders and growers, both of whom are striving to synchronise crop development with their target environment. The newly released variety Compass has demonstrated an average 10% yield advantage over the benchmark malting variety Commander across southern Australia. This is a large increase by commercial standards and essentially sets a new benchmark for future variety releases. However, the link between crop development and its improved yield is not known. The substantial yield improvement in Compass provides an opportunity to identify the pattern of development and the distribution of biomass that have resulted in its increase in yield potential.

In the temperate cereal production areas of Australia, climatic conditions in autumn define the periods for sowing and the phenological events that follow in winter and spring. The optimum flowering window is a compromise between minimising the risk of frost damage at ear emergence and avoiding high temperature and terminal water stress during grain filling. This

is achieved by an appropriate combination of photoperiod (Ppd) and low temperature vernalisation (Vrn) response genes, and earliness per se (Eps or Eam) loci (Campoli and von Korff, 2014; Cockram *et al.*, 2007). Of these developmental controls, Boyd et al. (2003) concluded that the best-adapted barley cultivars for Australian low-medium rainfall environments are early-maturing spring types that exhibit a relatively high photoperiod response and limited vernalisation. This has led to adapted cultivars that can be sown in May and rapidly progress to flower in spring when days became longer while vernalisation has not been a focus of spring barley breeding for southern Australia (Boyd *et al.*, 2003).

It is widely recognised that an increase in grain number is the dominant driver of cereal yield improvement in Mediterranean environments (Peltonen-Sainio et al., 2007), but malting quality also requires varieties to produce large, plump grain and both these traits are sensitive to the timing and duration of development leading up to flowering. Flowering time is a key adaptive trait; it is often already optimised in established breeding program (Slafer *et al.*, 2005) and the length of the total cycle is generally well adjusted for a particular environment (Isidro *et al.*, 2011). However, adjustment of pre-anthesis phases (vegetative and reproductive) independently to improve grain number without modifying total time to heading has been proposed as means for further yield improvement (Appleyard *et al.*, 1982; Kitchen and Rasmusson, 1983; Slafer *et al.*, 2005; Borras *et al.*, 2009).

The main selection criteria used by breeders for improved adaptation -flowering time and yield - implies indirect selection for changes in pre anthesis developmental phases and grain number. The timing and durations of the pre-anthesis developmental phases - the vegetative phase (floral initiation), the early reproductive phase (spikelet primordia initiation) and the late

reproductive phase (spike growth and development) - play critical roles in determining grain number. Following floral initiation, a period of spikelet initiation occurs up to the awn primordia stage, after which spikelet abortion occurs. The period of growth from awn primordia to tipping (spike growth phase) has been suggested to be the most critical for determining grain number. The high rate of spikelet and floret mortality during the late reproductive phase coincides with rapid growth of the stem and spike, causing competition for assimilates (Kirby, 1988; Fischer, 2007; Alqudah *et al.*, 2014).

Understanding of and variability in pre-anthesis development within Compass and elite cultivars that holds a mechanistic link with grain yield will be of particular importance for breeders aiming to fine tune crop phenology for improved adaptation. In Australia, broadly adapted barley varieties are favoured over varieties with narrow adaptation. This is partly due to the stringent market requirements for malting quality that delays new varieties being accepted for malting. Additionally, malt barley varieties are segregated individually beyond the farm gate, creating storage and logistics issues as grain handlers have a limited capacity to accept a large number of different varieties. Moreover, the large area over which barley is grown means that to be commercially successful, a variety needs to yield well over a range of environments and sowing dates. This requires a degree of developmental plasticity. Genetic control of a trait and of its plasticity may not be closely associated which suggests that different combinations of traits and their plasticity can be targeted (e.g. large seed size combined with low plasticity of seed size) (Sadras and Richards, 2014). Many studies in barley have focused on agronomically important traits but few on their plasticity. Therefore, this remains a research gap and a focus on trait means *per se* (such as crop yield) and the plasticity of phenology may be a useful approach to understand how adaptation can be improved.

The primary objective of the present study was to evaluate the pattern of pre-anthesis development contributing to yield improvement in the broadly-adapted cultivar Compass compared to the current benchmark variety Commander and other elite varieties. Compass is derived from Commander and is genetically similar. In this paper, the differences in yield physiology between contrasting barley cultivars grown under different growing conditions are reported with the aim to understand and evaluate the relevance of yield determining traits for adaptation to Mediterranean environments.

Materials/Methods

Phenology and yield component dataset:

Six experiments were conducted in South Australia between 2014 and 2015 at the Charlick Research Farm, Strathalbyn SA (35°19'19.9"S 138°53'02.5"E). In each year field trials were sown on three dates, four weeks apart: April 28, May 25 and June 21.

Experiments were sown at three planting dates in each year to expose genotypes to different photoperiod and temperature regimes. Rainfall, temperature, and daylength statistics are presented in Table 7. Day lengths, including civil twilight, were calculated using the formulae of (Forsythe *et al.*, 1995).

Table 7. Monthly and long-term weather statistics for the growing season at Strathalbyn, SA

	April	May	June	July	Aug	Sep	Oct	Nov	Mean (Apr - Nov)	Sum (Apr - Nov)
					Average Maximum temperature (°C)					
Mean	22.2	18.6	15.8	14.9	16.2	18.7	21.5	24.5	19.1	
2014	21.9	20.1	15.9	14.8	16.3	19.9	25.2	25.8	20.0	
2015	18.7	17.5	15.3	14.1	14.9	18.1	26.0	25.6	18.8	
					Average Minimum temperature (°C)					
Mean	10.5	9.2	7.4	6.5	6.6	7.7	8.8	11.3	8.5	
2014	11.8	10.7	9.1	7.6	5.5	7.9	10.2	11.7	9.3	
2015	9.9	9.2	7.3	5.6	6.7	6.5	10.3	12.4	8.5	
					Rainfall (mm)					
Mean	29.2	43.6	50.8	55.9	49.4	49.6	34.0	25.9		338.4
2014	28.2	49.4	65.6	63.6	22.6	20.2	9.4	11.0		270.0
2015	84.4	63.8	15.0	59.2	32.6	17.0	3.8	38.0		313.8
					Photoperiod (hrs)					
Mean	12.03	11.15	10.75	10.99	11.78	12.86	14.02	15.02		

In each experiment, 12 genotypes were sown: three high-yielding and widely-adapted spring varieties (Compass, Hindmarsh, and Fathom), three elite breeding lines that were full siblings of Compass (WI4895, WI4896, and WI4897), the parent of Compass and current benchmark cultivar for yield and malt quality (Commander), and five slow-developing cultivars Navigator, Admiral, Westminster, County (also a parent of Compass), and the winter cultivar Urambie.

The experiments were sown at a density of 150 seeds/m^2 and planted in plots 5 rows wide (21.5 cm row spacing) by 3.8 m long. The plots length was reduced to 3m prior to harvest. Each experiment received 75 kg ha^{-1} DAP (18:20: 0: 1) at sowing and was top-dressed with 46 kg N ha^{-1} (as urea - 46% N) during mid tillering. Weed management and disease control followed normal commercial practice using herbicides and fungicides at recommended rates and growth

and yield of the plots were not affected by disease or weed competition. Experiments were a split-plot, with sowing dates randomly allocated to whole plots and the cultivars to sub-plots with four replications. The trials were designed in a row-column design to allow for the spatial variation in yield to be accounted for in the analysis

Phenotypic measurements of phenology

From seedling emergence to heading (Zadoks growth stage Z59), four plants were randomly sampled every 3 to 14 days and the main stems dissected to examine the development of the apical meristem using the method of (Kirby and Appleyard, 1987b; Kirby and Appleyard, 1987a). Immature barley inflorescences were prepared for microscopic dissection and image capture. The floret developmental stage was determined using a modified version of the scale of Waddington *et al.* (1983) and the stages described in Kirby and Appleyard (1987a). External growth stages were described using Zadoks growth stages (Zadoks *et al.*, 1974). Primordia were counted to determine the maximum number of spikelet primordia initiated. Dissection of the main stem stopped once a genotype had reached the start of awn primordium - Waddington scale of 4.5 (W4.5) - and sampling was commenced again at flag leaf sheath extension (Z 39-41). At anthesis (Z 65), the total number of fertile florets (stage W10) on the main stem spikes was determined on 10 plants.

The key development stages determined were; W2, corresponding to the appearance of double ridges (DR) on the apical meristem; W 4.5, which is awn primordia (AP) and typically occurs between Zadoks 31-33 and is deemed to represent the time of maximum primordia number; and W10, as the phase in which 50% of the florets in the main spike are at anthesis (Z 65). Based on this analysis the following four phases were defined:

(i) Vegetative phase: time from sowing to DR (W2).

(ii) Spike initiation phase (SI): time from DR (W2) to AP (W4.5).

(iii) Spike growth phase (SG) time from AP (W4.5) to Anthesis (W10).

(iv) Pre-anthesis: the time from sowing to anthesis (W10).

The duration of each phase was measured in days and in thermal time units (°Cd, degree days) using 0 °C as base temperature (Kirby, 1988). Sampling did not always allow the time of a specific growth stage to be captured on the day of sampling and so time was estimated from interpolation by fitting a linear or polynomial regression of the Waddington developmental scores against accumulated degree days and days from sowing.

Yield and yield components:

Total above-ground biomass at maturity and yield components were estimated from a quadrat sample totalling 1m of row per plot. Plants were cut at ground level and the number of spikes counted. Spikelet number per spike was counted on a subsample of 50 randomly selected spikes. The spikes from the quadrat sample were threshed by hand and weighed. Harvest index (HI) was calculated as the ratio of grain weight to total biomass. The grain weight of 1000 grains (TGW) was measured at harvest from the plot grain sample. Grain yield was measured by harvesting each plot using a Wintersteiger small plot harvester. Spikelet survival and spikelet fertility were estimated using the following formulae:

$$Spikelet\ survival\ (\%) = \frac{Mature\ spikelets\ at\ anthesis}{Spikelets\ at\ awn\ primordia\ initiation}\ x\ 100\%$$

$$Spikelet\ fertility\ (\%) = \frac{Grains\ per\ spike\ at\ maturity}{Mature\ spikelets\ per\ spike\ at\ maturity}\ x\ 100\%$$

National Variety Trial dataset

Each year a series of National Variety Trials (NVT) are planted throughout the cereal zone and yield data are collected. Grain yield information was obtained from 49 NVTs conducted in

South Australia and Victoria between 2014 and 2016. Fifteen cultivars were chosen including the latest-maturing cultivars Westminster and Oxford, early-maturing cultivars Hindmarsh, Fathom, Compass, and the benchmark Commander along with LaTrobe, Scope, Spartacus CL, Rosalind, Bass, and Flinders. Sites were only included if they contained all 15 cultivars. This meant that, across years, the data were balanced and each genotype was grown every year, however, not all locations were represented for each year (Table 8).

Table 8. Summary statistics for the NVT trials sites located throughout South Australia and Victoria

NVT analysis	Year			
	2014	2015	2016	Mean
Number of sites	19	18	12	
Grain yield (kg/ha)				
Minimum	1370	1260	3960	1250
Maximum	5660	4800	7260	7260
Mean (± SD)	4000 ± 1.05	2870 ± 1110	6030 ± 1020	4080 ± 1610
Compass mean	4110	3360	5290	4260
Commander mean	3680	2850	5450	3900

Statistical analyses

Yield and phenology traits were analysed with ANOVA using the statistical package GenStat (18th ed.) and significance was tested using a post hoc Tukey HSD at probability level $P < 0.05$. The GxE interaction was tested in ANOVA with each sowing date and site combination considered a different growing condition (E factor), equating to six environments and twelve genotypes.

Regression analysis and Pearson phenotypic correlation analyses among genotypes and growing conditions were calculated for selected traits and figures were produced using GraphPad Prism 8. In some instances to illustrate general relationships between the components of yield across experiments and minimise the impact of environment, each variable was calculated as a value relative to the average of each experiment as outlined by Slafer *et al.* (2014).

Principal component analysis (PCA) was used to interpret and summarise the major pattern of variation among growing conditions and genotypes by phenology measurements, yield, and yield components. PCA was calculated based on genotype means for each trait under each growing condition, to study the inter-relationships among the components using the Unscrambler software (version 10.3, CAMO, Norway). Means were standardised using 1/SD in order to account for the effect of scale and were conducted on covariance matrices.

Phenotypic plasticity from the phenotypic data and the NVT yield data was calculated as a variance ratio (Dingemanse *et al.*, 2010) and the relationships between the plasticity of the trait for each cultivar and the maximum and minimum values examined using least squares regression. For phenological traits, the minimum values correspond to late sowing (June) and the maximum values for April sowing, whereas for the NVT data set the 1st and 9th percentiles were used to represent unfavourable and favourable conditions.

Results:

Seasonal conditions

In 2014 and 2015, total growing season rainfall was less and the distribution different from the long-term average. Most in-season rain fell during Apr to July consistent with long-term weather patterns however; this was followed by below average dry periods in both 2014 and 2015 during August – October corresponding to anthesis and grain filling. Minimum

temperatures were milder in April – July 2014 compared to the long-term averages and to 2015. Maximum temperatures were generally consistent with long-term trends. Frost damage was negligible in these environments (Table 7). Photoperiods declined from April until Jun and began to increase thereafter.

Variation in phenology and yield

Averaged across 2014 and 2015 the thermal time to anthesis declined from 1576 °Cd with April sowing, to 1443 °Cd at May sowing, and 1285 °Cd with June sowing. Within environments there was significant genotypic variation for time to anthesis but there were two clear development classes based on their mean time to anthesis (Figure 9): slow developing cultivars (Admiral, County, Navigator, Urambie, Westminster), Commander and fast developing spring cultivars (Compass, Hindmarsh, Fathom, WI4895, WI4896, and WI4897).

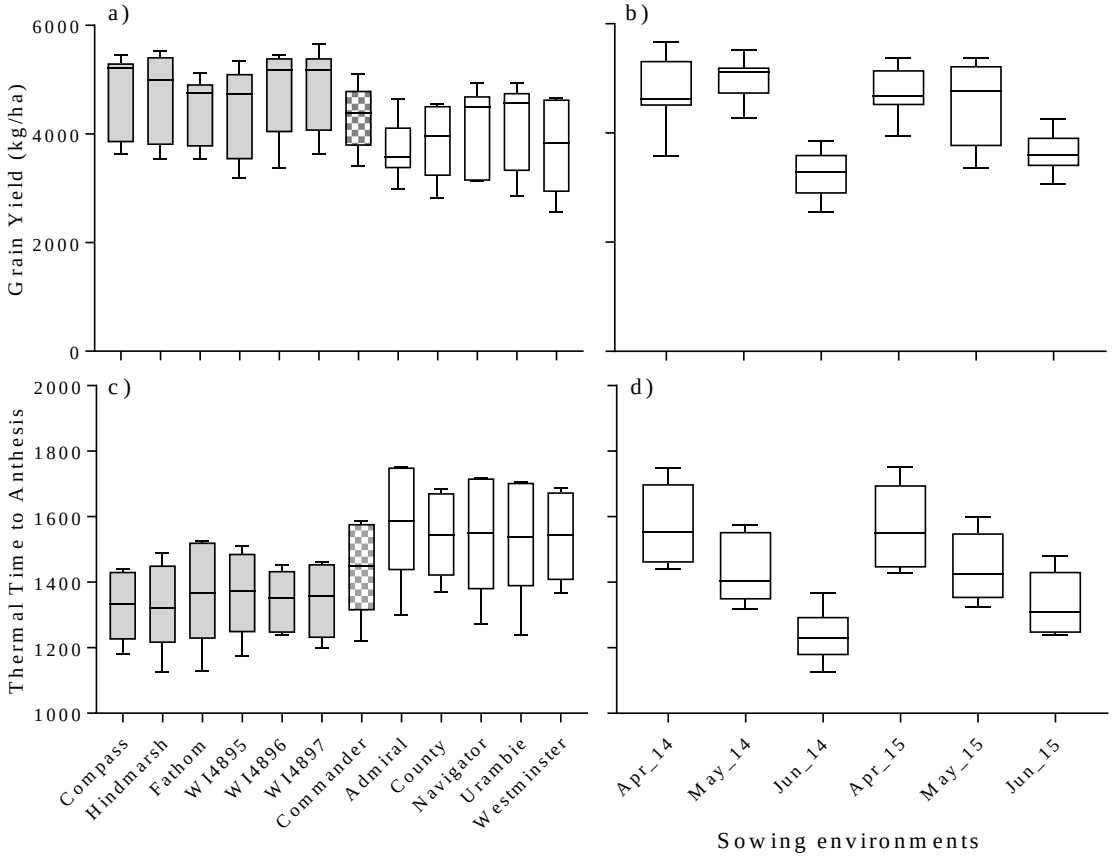


Figure 9 Boxplot summary for grain yield (a, b) and thermal time to anthesis (c, d) averaged for all genotypes (a, c) and environments (b, d). Shaded boxes represent the fast developing cultivar group and open boxes the slower developing spring genotypes, Commander is pattern shaded differently as the reference cultivar.

Across environments and cultivars, grain yields ranged from 2557 to 5661 kg/ha (Figure 9). ANOVA revealed significant GxE interactions for grain yield, grain number, grains per spike, and spike number (Table 9). Exploration of GxE for grain yield in the PCA output (Supplementary Figure 3) illustrated the strong influence of developmental group and sowing date on the yield results with variation in PC1 correlated to sowing date in both 2014 and 2015 and genotypes in PC2 were clustered relative to their maturity. It was concluded that the GxE interaction for grain yield was mainly due to sowing date and anthesis date within environments rather than season (Supplementary Figure 4). This supports the use of ANOVA for analysis of

genotype means across environments or within similar sowing times particularly as Compass WI4896, and WI4897 were the equal highest yielding varieties within each environment and other cultivars were subject to more GxE. The current benchmark variety Commander represented intermediate yield and time to flower in all environments.

Variation in yield and components

Compass, yielded 14.1% higher than Commander with April sowing, 10.1% with May, and 4.5% at June sowing. On average Compass had 10,827 (± 734) grain m^{-2}, 22% fewer grains m^{-2} than Hindmarsh at 12,391 (± 630) and 7% more than Fathom (10,099 ± 970). Grain weight was less influenced by environment than grain number. Fathom, Compass, and WI siblings produced larger grains than Commander and all other varieties across all sowing environments (Supplementary Figure 8). On average the grain weight of Fathom (46.4 ± 0.5) was higher than Compass (44.9 ±0.7) and Hindmarsh (38.7 ± 0.7 mg). Compass achieved more grains per spike than Commander did in May and June sowing environments but a similar number of spikes per m^2. Other cultivars Hindmarsh, Admiral and Navigator produced significantly more spike m^{-2} than Compass in all environments and Admiral and Navigator fewer grains per spike (Supplementary Figure 9). Significant variation in biomass and HI was obtained across genotypes and environments; however, there was not any GxE interaction for either measurement. The average biomass at maturity ranged from 867 g m^{-2} in Admiral to 1073 g m^{-2} in WI4895. Compass achieved both more biomass and higher HI than Commander did, and all of the fast developing lines consistently produced more biomass than the slow developing lines. The biomass of Compass was 1031 g m^{-2} with a HI of 47%, whereas Hindmarsh produced slightly less biomass (980 g m^{-2}) at a similar HI (Table 9).

Table 9. Summary of genotypes means for grain yield and measures of yield components, biomass and harvest index across all environments and summary of significance for ANOVA output. # indicates fast developing genotypes. Statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and NS ($P > 0.05$). The same letters are not significantly different at $P \leq 0.05$.

	Grain Yield (kg/ha)		Grains per m^2 ($\times 10^{-3}$)		Grain Weight (mg)		Grains per spike		Spikes per m^2		Biomass at Maturity ($g\ m^{-2}$)		Harvest Index	
Admiral	3705	a	10.23	bc	38.0	a	13.9	a	733	e	867	a	0.440	a
Westminster	3760	a	9.39	a	42.2	c	18.9	f	500	a	899	ab	0.435	a
County	3864	ab	10.12	bc	39.4	b	17.5	cde	574	bc	899	ab	0.428	a
Navigator	4136	bc	10.99	e	39.1	b	16.8	bc	646	d	931	bc	0.449	abc
Urambie	4181	c	10.22	bcd	41.8	c	19.1	f	539	ab	928	abc	0.452	abc
Commander	4316	cd	10.22	b-e	42.1	c	17.2	cd	592	c	957	bc	0.446	ab
WI4895#	4447	de	10.08	b	45.7	e	15.8	b	646	d	1074	e	0.432	a
Fathom#	4477	def	10.10	ab	46.7	f	18.4	ef	567	bc	1062	e	0.458	abc
Hindmarsh#	4716	efg	12.40	f	38.6	ab	17.4	cde	722	e	981	cd	0.474	c
Compass#	4779	fg	10.83	cde	44.9	de	18.3	ef	599	cd	1031	de	0.473	c
WI4896#	4802	g	10.87	de	45.0	de	18.5	ef	600	cd	1035	de	0.462	bc
WI4897#	4864	g	10.97	e	44.5	d	18.2	def	606	cd	1030	de	0.462	bc
Mean	4337		10.53		42.3		17.5		610		974		0.45	
G	***		***		***		***		***		***		***	
E	***		**		***		***		***		**		NS.	
G x E	*		***		NS.		***		***		NS.		NS.	

Developmental traits

There was a significant genotype x sowing date interaction for time to DR, time to awn primordia, and time to anthesis and no interaction for the duration of the spike initiation and spike growth phases (Figure 10). Compass was the fastest to anthesis in all environments and in comparison to Commander is was also faster to awn primordia. From April sowing Compass, WI4896, WI4897, and Hindmarsh all reached double ridge and anthesis at a similar time; however, Compass and WI4896 reached awn primordia earlier than Hindmarsh. Commander was longer to DR, awn primordia, and anthesis (Figure 10a). Compass, Hindmarsh, WI4896 and WI4897 were the fastest developing with May sowing, reaching double ridge, awn primordia and anthesis at similar times. Commander reached double ridge at a similar time to Compass but was later to awn primordia and anthesis than other fast developing genotypes but earlier than the slow developing genotypes (Figure 10b).

When sown in June, Compass reached anthesis at a similar time to all the fast-developing genotypes and Commander. However, Compass was later to reach double ridge than Fathom and Hindmarsh and similar to WI4897, WI4895, WI4896, and Commander. Compass was also quicker to awn primordia than Commander (Figure 10c).

The timing and the duration of pre-anthesis phases were all strongly correlated with the time to flower (Supplementary Table 4). However, within each sowing environment there is evidence of cultivars flowering at a similar time with different combinations in the duration of pre-anthesis phases. For example, Fathom was similar to WI4896 and WI4897 in time to double ridge at April and May sowing but had a longer duration to awn primordia. Within slow developing lines, Urambie was later to double ridge and awn primordia but flowered at a similar time to Westminster. With June sowing, all slow-developing lines flowered at a similar time but County was the slowest in development in all pre-anthesis phases (Figure 10).

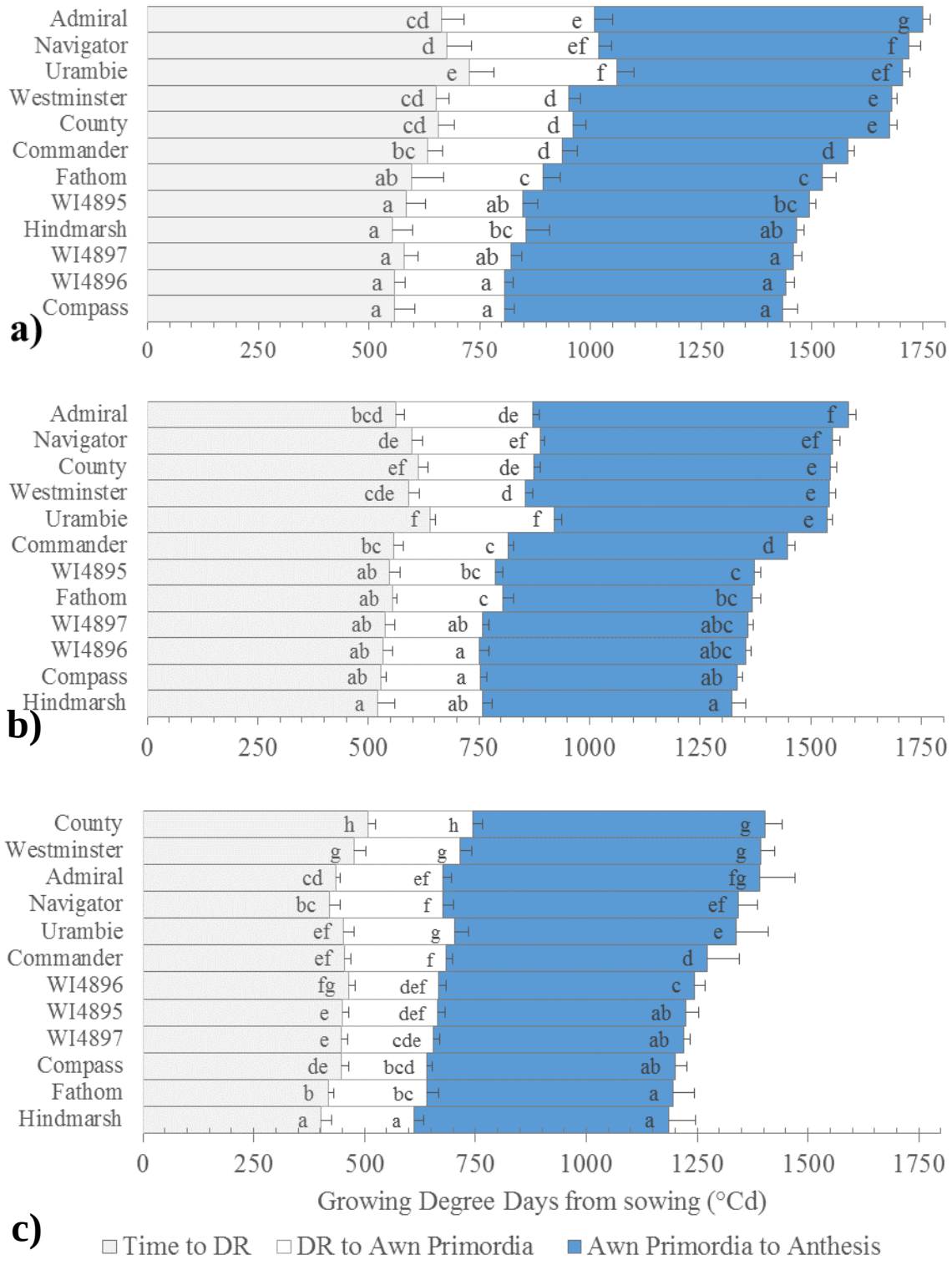


Figure 10. Mean duration in the total thermal time from sowing to either double ridge (DR), awn primordia, and anthesis across the three sowing environments (a) April sowing, (b) May sowing, and (c) June sowing. Error bars represent the standard error of the mean n = 2. ANOVA for all three measurements have genotype x sowing date interaction (Fpr=<0.001). The same letters are not significantly different at $P \leq 0.05$ within each sowing date and trait.

The duration of spike initiation and spike growth phases were subject to a smaller GxE effect and were not as strongly correlated with flowering time as the other phases (Supplementary Table 4 and Supplementary Table 4). On average Compass and its siblings had a shorter spike initiation phase than other genotypes but a similar duration of the spike growth phase compared to the other fast developing cultivars Hindmarsh and Fathom. Commander had a similar spike initiation duration to Hindmarsh, Fathom, County, and Westminster but all varieties had different spike growth durations. This means Compass, WI4897 and WI4896 spent less of their reproductive phase in the spike initiation phase (Table 10). Fathom initiated the most spikelet primordia, (on average 50.5 per main spike) compared to Compass (42.1). However Compass, WI4896, and WI4897 had a higher spikelet survival, and while Fathom had poor spikelet survival it still achieved the highest number of grains per main spike (23.2).

Table 10. Summary of ANOVA genotypic means for duration of spike initiation and spike growth, maximum spikelet primordia number, mature spikelets at anthesis, mature spikelets at maturity, spikelet survival (%), and spikelet fertility (%) on the main spike across all environments. Statistical significance is **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and NS (*P* > 0.05). Same letters are not significantly different.

	Duration of Spike initiation (°Cd)		Duration of Spike Growth (°Cd)		Max Spikelet Primordia per main spike	Mature spikelets per main spike at Anthesis	Mature spikelets per main spike at Maturity	Spikelet Survival (%)	Spikelet Fertility (%)
Admiral	299	e	724	e	41.8	16.7	16	40.1	95.4
Commander	260	c	641	c	41.8	20.3	19.3	48.6	94.6
Compass	227	a	594	a	42	21.2	20.6	50.5	97.1
County	267	c	682	d	43.5	21.6	20.2	49.7	93.3
Fathom	257	bc	583	a	50.5	23.2	22.5	46.2	97.2
Hindmarsh	250	bc	583	a	42.7	21	20.3	49.3	96.6
Navigator	296	e	676	d	44.9	20.1	18.9	45	93.6
Urambie	287	de	632	bc	47.8	23.4	22.4	49.1	96
Westminster	274	cd	696	de	47	23.3	21.7	49.6	92.9
WI4895	239	ab	598	a	41.5	21.7	19.5	52.3	90
WI4896	223	a	605	ab	40.9	21.9	21.1	53.4	96.4
WI4897	223	a	600	a	41.3	21.1	20.3	51.1	95.9
F.Pr	<0.001		<0.001		<0.001	<0.001	<0.001	<0.001	<0.001
LSD					1.8	0.63	0.7	1.78	1.49

Linking development to grain yield

To minimise the impact of environment, each variable was calculated as a value relative to the average of each experiment as outlined by Slafer *et al.* (2014). Slow rates of development were consistently associated with lower relative yields at each SD (Table 11). Maximum primordia number was not associated with grain yield however, spikelet survival was associated with high yields, along with post anthesis traits biomass and HI, which infers C and/or N allocation, may be important. Higher relative grain yield was associated with more grains m^{-2} and with higher grain weight. More grains per spike was associated with high yields at May sowing. No relationship was found between grain yield and the sub component spikes per m^2.

Table 11. Pearson correlations between grain yield, measures of crop phenology and yield components form the mean of 2014 and 2015. Correlations are on based on genotype values relative to the mean of all genotypes within each sowing date and the data from the two years have been combined. Statistical significance is **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and NS (*P* > 0.05). Statistical significance is **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and NS (*P* > 0.05).

	Sowing date					
	April		May		June	
Time to DR	-0.68	***	-0.53	*	-0.33	NS
Duration Spike Initiation	-0.84	***	-0.59	*	-0.76	***
Duration Spike Growth	-0.66	***	-0.78	***	-0.75	***
Time to Awn Primordia	-0.81	***	-0.63	**	-0.70	***
Time to Anthesis	-0.84	***	-0.76	***	-0.80	***
Max Primordia number	-0.16	NS	-0.38	NS	-0.29	NS
Spikelet Survival	0.55	**	0.76	***	0.54	**
Grain per Spike	0.32	NS	0.52	**	0.35	NS
Grain Weight	0.63	***	0.27	NS	0.54	**
Spikes per m^2	-0.09	NS	0.23	NS	0.20	NS
Grain Number m^2	0.57	**	0.68	***	0.80	***
Biomass	0.70	***	0.81	***	0.80	***
Height	0.44	*	0.37	NS	0.33	NS
Harvest Index	0.59	**	0.68	***	0.65	**

Across all environments, shorter development phases were associated with higher relative grain weight. Beyond grain yield and grain weight there were few strong correlations between duration and timing of pre-anthesis phases, and the subcomponents of grain number and spikelet survival (Supplementary Table 4). A greater spikelet survival led to more grains per spike and a higher HI; however, more spikes per m^2 led to fewer grains per spike and reduced spikelet survival. As a result, high yielding genotypes combined different yield components. The PCA for May sowing highlights the different genotypic combinations of all the variables. The majority of the variation was explained in PC1 and 2 and all variables related to yield such as spike per m^2, grains per spike and HI provided greater differentiation between genotypes than developmental traits. The complementary PCA plots for April and June can be found in Supplementary Figure 5 and Supplementary Figure 6.

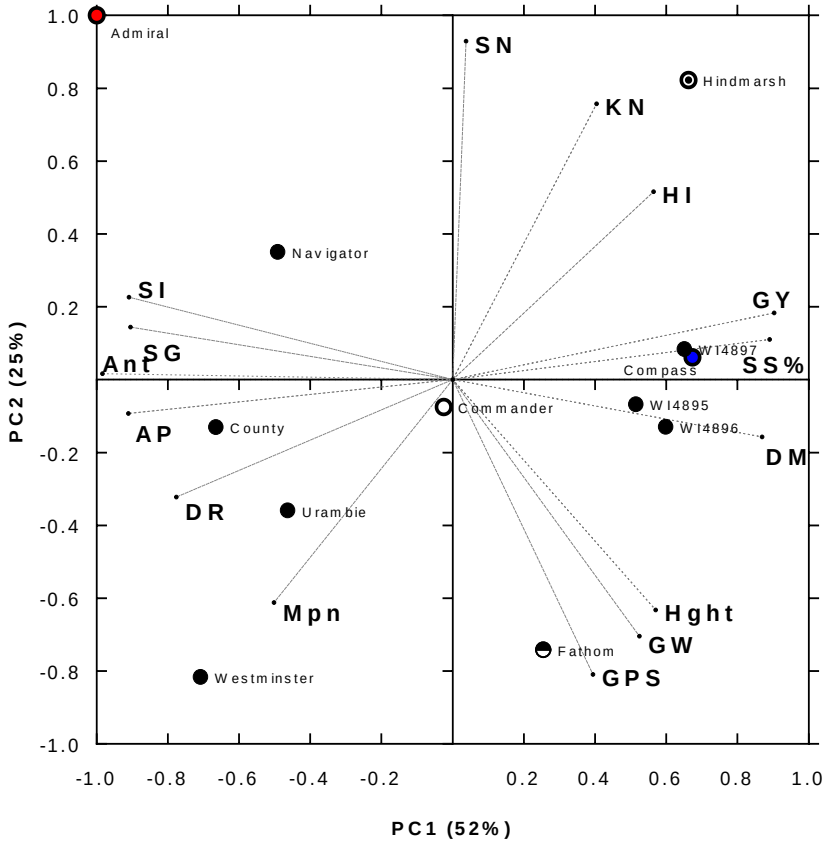


Figure 11 Biplot combining the PCA scores and Loadings for May sowing genotypic variability prevailing in the 12 genotypes in terms of Dry Matter (DM), Spike per m2 (SN),

Grain Number (KN), Grain Weight (GW), Grain Yield (GY), Height (Hght), Maximum Primordia number (Mpn), Spikelet Survival (SS%), Grains per spike (GPS), Time to Double Ridge (DR), Time to Awn Primordia (AP), Time to Anthesis (Ant), Duration Spike Initiation (SI), Duration of critical phase (SG)

The patterns observed in the PCA plots show compared to Commander and other long season cultivars, the improved yield of Compass and siblings WI4896, WI4897 has come from a combination of changes in many small traits, mainly by shortening all phenological phases, a slight increase in dry matter, HI, grain number, and consistently larger grain weight. The other high yielding line's Hindmarsh and Fathom had different phenology and yield structures. Compared to Compass Hindmarsh was defined by a relatively greater grain number resulting from greater spikes per m^2 but a trade off in grain weight and the variety Fathom greater grains per spike and grain weight (Figure 12).

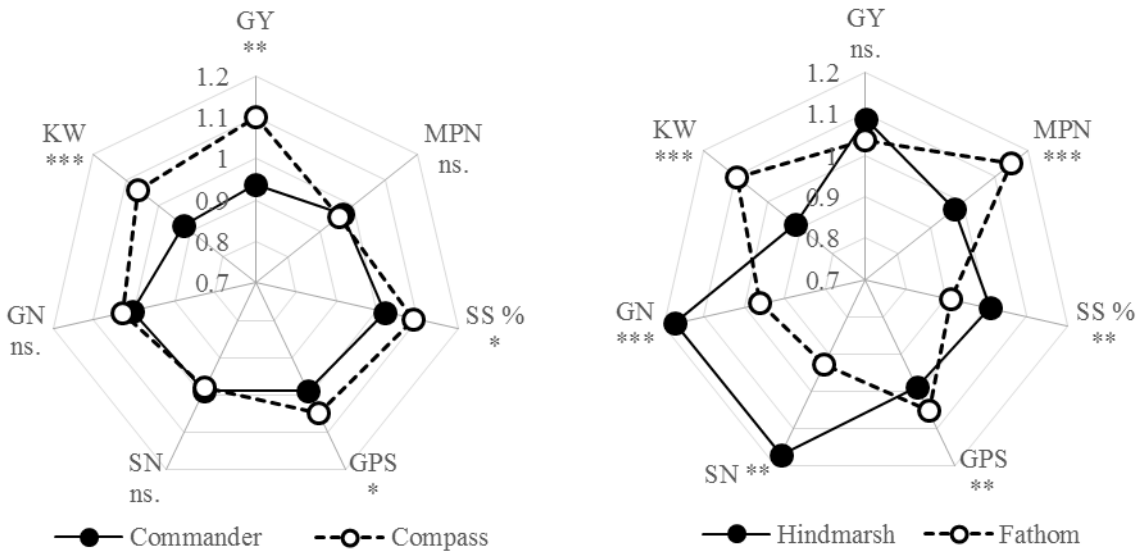


Figure 12. Radar charts comparing the traits of yield and yield components of a) Commander and Compass, b) Hindmarsh and Fathom. Six individual environments were used in each of the measurements and the data subjected to one way ANOVA followed by Students t-test Statistical significance is $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ and NS ($P > 0.05$). Data are relative to the mean (1.0) of each experiment and include both genotypic and environmental factors. Grain Yield (GY), Maximum Primordia Number (MPN), Spikelet Survival (SS%), Grains per spike (GPS), Spike per m2 (SN), Grain Number (GN), Grain Weight (KW).

Plasticity of phenology and yield

Phenological plasticity was not strongly associated with yield plasticity nor with yield in favourable conditions (early sowing) or unfavourable conditions (delayed sowing) (Table 12). When the data was separated into fast and slow developing cultivar groups, similar conclusions were made. Irrespective of plasticity, fast developing lines were higher yielding in both the high and low yielding environments.

Table 12. Correlation coefficients between plasticity of yield, April and June sowing yield of barley. Statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and NS ($P > 0.05$).

	Plasticity of Yield		April Sowing		June Sowing	
Plasticity of yield	-		NS		NS	
Plasticity of Grain number	NS		NS		NS	
Plasticity of Grain weight	NS		NS		NS	
Plasticity of Spike per m^2	NS		NS		0.43	*
Plasticity of grains per spike	NS		-0.49	*	NS	
Plasticity of sowing to anthesis duration	NS		-0.42	*	-0.58	*
Plasticity of sowing to double ridge duration	NS		NS		-0.50	*
Plasticity of sowing to awn primordia duration	NS		NS		-0.56	*
Plasticity of duration of critical phase	-0.45	*	NS		NS	
Plasticity of critical phase fraction	-0.47	*	NS		NS	

To expand the investigation, the same plasticity framework was applied to the NVT dataset. Across the 49 experiments used in the NVT, dataset average site grain yields of all genotypes ranged from 1260 kg/ha to 7260 kg/ha (Table 8). The NVT dataset showed similar trends to the phenology experiment and particularly Compass was less plastic compared to slower developing lines resulting in higher yields under stressed conditions (10^{th} percentile). However,

this was not associated with a yield trade-off in more favourable environments because Compass produced yields similar to lines with greater plasticity. In this dataset, there was little evidence to suggest cultivars with high plasticity (i.e. greater than 1) may yield more than varieties with less plasticity in both favourable and unfavourable environments (Figure 13).

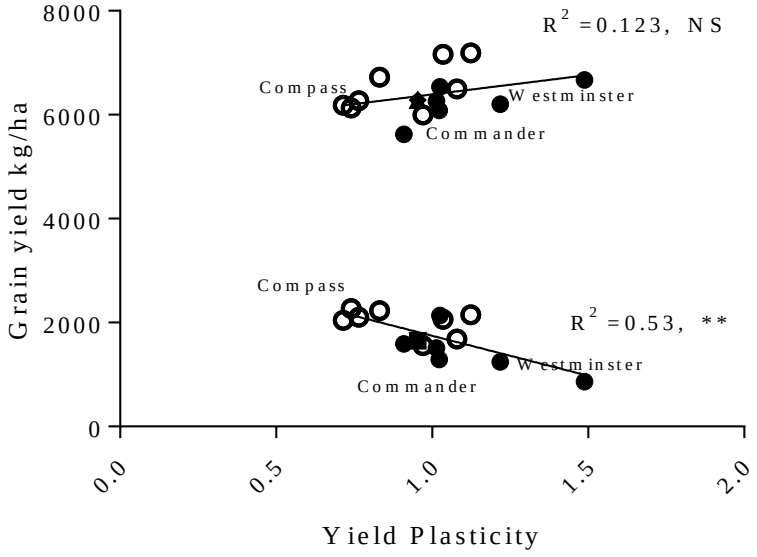


Figure 13. The relationship between yield plasticity and the 10th and 90th grain yield percentile in 15 genotypes across 49 NVT trials. Slow (closed symbols), and fast (open symbols) developing genotypes.

Discussion:

Yield and phenology

While the analysis of yield and phenology revealed some GxE interaction for grain yield due to sowing date, this was negligible particularly in the context of this study as Compass was the highest yielding cultivar in all environments. The substantial and stable yield improvement in Compass provides an opportunity to identify the pattern of development and the distribution of biomass that have resulted in its increase in yield potential. Of all the phenology measurements, the time to anthesis date was the most strongly correlated with grain yield, consistent with findings in most Mediterranean environments (Shepherd *et al.*, 1987). Faster

developing genotypes were better adapted to southern Australian environments when sown at current commercial planting times. However, it should be mentioned these results are derived from a field experiment located in an environment not prone to reproductive frosts; this was deliberate in order to look at yield potential across sowing environments. Yields of early-flowering genotypes may differ in other environments due to frost particularly with April sowing times. However, our yield data confirm the results from the NVT trials where Compass and Hindmarsh have been among the equal highest yielding cultivars and reflects yield performance in low rainfall Mediterranean environments of southern Australia sown at conventional sowing dates and commercial frost prone farming environments in southern Australia.

Breeding for yield and its components?

Improvements in grain yield came from an increase in grains m^2 and grain weight within faster developing varieties. Grain weight was more stable among genotypes and sowing conditions. Compared to Commander, breeders have achieved a yield increase in the cultivar Compass involving similar or modest improvements in grain number with consistently heavier grain. Grain number, the dominant factor for grain yield, was driven by varying combinations of spikes m^2 and grains per spike. The lack of any clear and consistent correlation between grain number components grains per spike and spike number and yield highlights the dynamic nature of yield formation in two-row barley. Grains per spike is only one contributor to grain number per m^2 and its association with grain number was weaker than the grain number association with spikes m^{-2}, as also reported by Arisnabarreta and Miralles (2006). A strong association with spikes m^{-2} in barley is explained by a high tillering capacity, (Arisnabarreta and Miralles, 2006; Arisnabarreta and Miralles, 2015; Bingham *et al.*, 2007). Previous studies have shown two-rowed barley cultivars possess a greater ability to establish fertile tillers than six-rowed barley cultivars (Kirby and Riggs, 1978; Peltonen-Sainio *et al.*, 2009), which have greater spike

plasticity when establishing grains per spike; therefore different strategies are required to establish yield in both barley spike types (Arisnabarreta and Miralles, 2008). This is also true within two row barley types. Depending on genotype, there was evidence of different yield structures. Among the high yielding cultivars, Hindmarsh had a consistently high grain number (mainly driven by spikes per m^2) and smaller grain, and Fathom has slightly more grains per spike and less spikes per m^2 but superior grain weight. Due to the dynamic nature of yield components, direct selection for pre-anthesis phases may be a more useful approach than targeted selection of yield components if a clear mechanistic link between pre-anthesis phases and grain yield can be established.

Variation in pre anthesis patterns and the link with grain yield

Compass provides an opportunity to identify the pattern of development and the distribution of biomass that have resulted in its increase in yield potential. It was hypothesised that partitioning of the pre-anthesis phases of Compass gives its yield advantage. Compass was the fastest to anthesis in all environments and in comparison to Commander is also faster to awn primordia and double ridge. However the length of the spike initiation phase showed some partial independence from the spike growth phase, where Compass, WI4896 and WI4897 spend less of their reproductive phase in the spike initiation phase compared to other cultivars.

Other studies have proposed that lengthening the period from awn primordia to tipping (spike growth phase) with differing phenology genes is promising for improving yield through increased spikelet development and survival (Alqudah and Schnurbusch, 2014). This would require either earlier sowing or a reduction in the length of the vegetative phase to ensure flowering occurs at the optimum time. Our results confirm spikelet survival was an important trait, as it correlated with more grains per spike and high grain yield. For this reason, improvements in spikelet survival remain realistic targets for yield improvement. However,

contrary to the literature spikelet survival was very poorly associated with the duration and timing of any pre-anthesis phases.

Grains per spike result from a complex process that involves a large overproduction of primordia occurring through the spikelet initiation phases. Only few primordia survive and form actual grains due to spikelet abortion during the spike growth phase. The influence of the duration of the spikelet initiation phase has often been neglected in the literature. Meanwhile, it has often been argued that extending the length of the spike growth phase may improve yield (Alqudah and Schnurbusch, 2014; Appleyard *et al.*, 1982; García *et al.*, 2011; Kernich *et al.*, 1997) rather than a shortening of the spike initiation phase.

In the present experiments, there is a very weak association between a shorter spike initiation phase and reduced maximum spikelet primordia number suggesting there is some trade-off in spikelet survival in cultivars that developed more spikelet primordia. However this trade off seems negligible as Compass has achieved similar maximum primordia number as other genotypes within a shorter spike initiation phase. This is important in the context of partitioning the pre anthesis phases, as it would appear that shortening of the spike initiation phase might not compromise yield potential, either through reduced number of maximum spikelet primordia or grains per spike. Therefore, it may be possible to continue to shorten the period of time from DR – Awn Primordia. Further research should focus on this phase and its effect on the development of spikelet primordia. An 'optimistic strategy' of generating more primordia than fertile florets could be a useful trait as the required investment involved in initiating primordia seems trivial (Fischer and Turner, 1978; Sadras and Slafer, 2012). For example, the genotype Fathom established the greatest number of spikelet primordia and, despite significantly reduced spikelet survival compared to other genotypes, still achieved the highest number of grains per spike in this study. Fathom achieved on average 50.5 grains per main spike; to our knowledge there are few examples in the literature where spring two-row

barleys have achieved >50 spikelet primordia, although this has been reported in six row barley (Alqudah and Schnurbusch, 2014; Kernich *et al.*, 1997). Targeting genotypes that have a high rate of spikelet primordia development may also be a fertile avenue to improve grain yield rather than targeting spikelet survival *per se*.

Despite conducting detailed measurements of pre-anthesis phases on well-adapted genotypes that differed in duration of the phases prior to anthesis, no clear mechanistic link with grain yield was found. In the case of Compass and Hindmarsh, improved yields were associated with shorter sub phases of development and contrasting yield structures. While there may be a biological limit to shortening development beyond Compass and Hindmarsh this finding would suggest that shorter durations and a more rapid rate of development is favoured and has in fact not limited yield potential. There is often commentary in the literature striving to identify optimal development patterns (Dofing, 1999), however these results demonstrate genotypes of similar time to anthesis can achieve similar yield outcomes through both different developmental patterns and through different sub components of grain number and grain weight means there are a number of pathways to achieve high yields. Therefore, it could be argued the selection for changes in specific phenological phases to improve yield may also be unsuccessful. The utility of this information for selection in breeding programs for improved yield may be negligible; correlations between components constrain their predictive value due to trade-offs and compensation between components in response to environmental variables (Sadras and Denison, 2009; Slafer and Rawson, 1994) . This makes breeding for grain number almost as complex as grain yield itself and could help explain why shifts in pre-anthesis phases may not relate to grain yield. Therefore, the idea of manipulating pre-anthesis for improved yield may have limited success.

However, breeders could consider selection from earlier sowing dates to exploit greater diversity, particularly as sowing date had a significant influence on the timing of the

phenological phases, time to double ridge and time to awn primordia. Our experiments highlight that there is more variation among genotypes for duration of the pre -anthesis phases from earlier sowing environments. Therefore, there appears greater possibility to alter the length and timing of these phases by manipulating the genes associated with sensitivity to environmental cues during the pre-anthesis period (Borras-Gelonch *et al.*, 2012; Borras-Gelonch *et al.*, 2010). The length of the spike initiation phase was not as closely related to the time to double ridge compared to other phenological phases suggesting there is opportunity for different phenological combinations of sub-phases when reaching the same time to anthesis. In general, there was less variation in phenological phase timing and duration in the faster developing lines compared to slow developing lines, which may be due to a lack of diversity in phenology genes and the fact the majority of variation in development among faster developing adapted Australian varieties has been associated largely with photoperiod sensitivity (Boyd *et al.*, 2003). This is a potential limitation in Australian germplasm and there is opportunity to introduce variation in vernalisation genes to adjust phenology patterns, particularly the phase from sowing to double ridge. For example, Urambie a winter cultivar requiring vernalisation was later to double ridge and awn primordia but flowered at a similar time to Westminster. Urambie and other slow developing varieties may offer a more appropriate flowering date from early sowing in April than Compass and fast developing varieties in frost prone environments. However, either they failed to achieve the same amount of biomass as Compass or had a lower harvest index compared to Compass sown in May.

Compass achieved larger grain weight, greater biomass and harvest index than slower developing lines. Although larger grain weight was associated with faster development and greater biomass, the causes of the improved grain weight of Compass was unclear and not obviously explained by the climatic conditions. Grain fill conditions were favourable from April sowing therefore this should favour slower developing cultivars to achieve a similar grain

weight as Compass, however this was not the case, suggesting the larger grain weight of Compass maybe due to factors other than phenology. The lack of a significant relationship between phenology and spikelet survival suggests that factors other than phenology may be more important in determining spikelet survival, particularly as the number of spikes/m^2 were negatively correlated with spikelet survival suggesting that competition for resources or source-sink relationships during the critical period is an important trait rather than the length of time. The relationship between biomass, yield and grain numbers tends to support this theory. Other mechanisms such as source-sink relationships and biomass partitioning may provide more scope for improved yield.

Yield improvement and plasticity

Selection for phenotypic plasticity has been proposed as a method for breeders to improve yield and adaptation (Sadras and Slafer, 2012), however the associations between phenological and yield plasticity traits with grain yield improvement or crop responsiveness to favourable conditions (early sowing) or unfavourable conditions (delayed sowing) were weak in our data. While these results are from limited environments and may not reflect all conditions experienced more widely across the southern barley growing region, there was supporting evidence from the NVT data that cultivars with higher plasticity than Compass and faster developing cultivars may have lower yields in lower yielding environments, whereas in higher yielding environments this trend was less pronounced. Nonetheless, in both analyses the fast-developing spring barley cultivar Compass combined higher or similar maximum grain yields and improved yield performance under low and high yielding conditions compared to slow developing cultivars with the same level of plasticity (Figure 13). This implies that plant breeders have been successful in improving yield stability along with yield potential within a shorter development cycle. This is of importance in the context of the variable growing conditions experienced in Australia and validates that selection for traits *per se* such as mean

duration to anthesis and yield over multiple environments remains an effective strategy for continual yield improvement.

Conclusion:

The release of the barley variety Compass represents a substantial improvement in the yield potential of barley adapted to southern Australia compared to the current benchmark Commander and other longer season cultivars. The results from this study demonstrate the increase yield of Compass is due to a combination of small changes in many traits, mainly by shortening all phenological phases, slight increase in dry matter, harvest index, grain number, and consistently larger grain weight. The findings in this paper shed light on the variation in phenology and the pre-anthesis phases in barley cultivars adapted to Southern Australia. We conclude that a dual focus on direct selection for an appropriate flowering time and yield remains one of the most effective approaches to optimise development patterns and the dynamics of grain yield. Particularly when barley appears to be very dynamic and adapted cultivars have a unique ability to compensate yield components and distribute assimilates into yield through multiple pathways. Future research to investigate the physiological and genetic basis of yield will focus on a large mapping population derived from a cross between Commander and Compass.

References:

Alqudah, A. M. & Schnurbusch, T. (2014). Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. *Functional Plant Biology* 41(4): 424-436.

Alqudah, A. M., Sharma, R., Pasam, R. K., Graner, A., Kilian, B. & Schnurbusch, T. (2014). Genetic Dissection of Photoperiod Response Based on GWAS of Pre-Anthesis Phase Duration in Spring Barley. *PLOS ONE* 9(11): e113120.

Appleyard, M., Kirby, E. J. M. & Fellowes, G. (1982). Relationships between the duration of phases in the pre-anthesis life cycle of spring barley. *Australian Journal of Agricultural Research* 33(6): 917-925.

Arisnabarreta, S. & Miralles, D. J. (2006). Yield Responsiveness in Two- and Six-Rowed Barley Grown in Contrasting Nitrogen Environments. *Journal of Agronomy and Crop Science* 192(3): 178-185.

Arisnabarreta, S. & Miralles, D. J. (2008). Critical period for grain number establishment of near isogenic lines of two- and six-rowed barley. *Field Crops Research* 107(3): 196-202.

Arisnabarreta, S. & Miralles, D. J. (2015). Grain number determination under contrasting radiation and nitrogen conditions in 2-row and 6-row barleys. *Crop & Pasture Science* 66(5): 456-465.

Bingham, I. J., Blake, J., Foulkes, M. J. & Spink, J. (2007). Is barley yield in the UK sink limited?: I. Post-anthesis radiation interception, radiation-use efficiency and source–sink balance. *Field Crops Research* 101(2): 198-211.

Borras-Gelonch, G., Denti, M., Thomas, W. T. B. & Romagosa, I. (2012). Genetic control of pre-heading phases in the Steptoe x Morex barley population under different conditions of photoperiod and temperature. *Euphytica* 183(3): 303-321.

Borras-Gelonch, G., Slafer, G. A., Casas, A. M., van Eeuwijk, F. & Romagosa, I. (2010). Genetic control of pre-heading phases and other traits related to development in a double-haploid barley (Hordeum vulgare L.) population. *Field Crops Research* 119(1): 36-47.

Borras, G., Romagosa, I., van Eeuwijk, F. & Slafer, G. A. (2009). Genetic variability in duration of pre-heading phases and relationships with leaf appearance and tillering dynamics in a barley population. *Field Crops Research* 113(2): 95-104.

Boyd, W. J. R., Li, C. D., Grime, C. R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Kamali, M. R. J., Barr, A. R., Moody, D. B., Lance, R. C. M., Logue, S. J., Raman, H. & Readj, B. J. (2003). Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. *Australian Journal of Agricultural Research* 54(11-12): 1277-1301.

Campoli, C. & von Korff, M. (2014).Chapter Five - Genetic Control of Reproductive Development in Temperate Cereals. In *Advances in Botanical Research*, Vol. Volume 72, 131-158 (Ed F. Fabio). Academic Press.

Cockram, J., Jones, H., Leigh, F. J., O'Sullivan, D., Powell, W., Laurie, D. A. & Greenland, A. J. (2007). Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. *Journal of Experimental Botany* 58(6): 1231-1244.

Dingemanse, N. J., Kazem, A. J. N., Réale, D. & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology & Evolution* 25(2): 81-89.

Dofing, S. M. (1999). Optimum development patterns for northern-adapted barley. *Cereal Research Communications* 27(3): 289-292.

Fan, Y., Tian, M., Jing, Q., Tian, Z., Han, H., Jiang, D., Cao, W. & Dai, T. (2015). Winter night warming improves pre-anthesis crop growth and post-anthesis photosynthesis involved in grain yield of winter wheat (Triticum aestivum L.). *Field Crops Research* 178(0): 100-108.

Fischer, R. A. (2007). Understanding the physiological basis of yield potential in wheat. *Journal of Agricultural Science* 145: 99-113.

Forsythe, W. C., Rykiel, E. J., Stahl, R. S., Wu, H.-i. & Schoolfield, R. M. (1995). A model comparison for daylength as a function of latitude and day of year. *Ecological Modelling* 80(1): 87-95.

García, G. A., Serrago, R. A., Appendino, M. L., Lombardo, L. A., Vanzetti, L. S., Helguera, M. & Miralles, D. J. (2011). Variability of duration of pre-anthesis phases as a strategy for increasing wheat grain yield. *Field Crops Research* 124(3): 408-416.

Isidro, J., Álvaro, F., Royo, C., Villegas, D., Miralles, D. J. & García del Moral, L. F. (2011). Changes in duration of developmental phases of durum wheat caused by breeding in Spain and Italy during the 20th century and its impact on yield. *Annals of Botany* 107(8): 1355-1366.

Kernich, G. C., Halloran, G. M. & Flood, R. G. (1997). Variation in duration of pre-anthesis phases of development in barley (Hordeum vulgare). *Australian Journal of Agricultural Research* 48(1): 59-66.

Kirby, E. & Appleyard, M. (1987a). *Cereal development guide.* Stoneleigh, UK: NAC Cereal Unit.

Kirby, E. J. M. (1988). Analysis of leaf, stem and ear growth in wheat from terminal spikelet stage to anthesis. *Field Crops Research* 18(2-3): 127-140.

Kirby, E. J. M. & Appleyard, M. (1987b). *Cereal development guide.* Kenilworth, England: Arable Unit, National Agricultural Centre.

Kirby, E. J. M. & Riggs, T. J. (1978). Developmental consequences of two-row and six-row ear type in spring barley: 2. Shoot apex, leaf and tiller development. *The Journal of Agricultural Science* 91(1): 207-216.

Kitchen, B. M. & Rasmusson, D. C. (1983). Duration and inheritance of leaf initiation, spike initiation, and spike growth in barley. *Crop Science* 23(5): 939-943.

Peltonen-Sainio, P., Jauhiainen, L. & Laurila, I. P. (2009). Cereal yield trends in northern European conditions: Changes in yield potential and its realisation. *Field Crops Research* 110(1): 85-90.

Peltonen-Sainio, P., Kangas, A., Salo, Y. & Jauhiainen, L. (2007). Grain number dominates grain weight in temperate cereal yield determination: Evidence based on 30 years of multi-location trials. *Field Crops Research* 100(2–3): 179-188.

R A Fischer, a. & Turner, N. C. (1978). Plant Productivity in the Arid and Semiarid Zones. *Annual Review of Plant Physiology* 29(1): 277-317.

Richards, R. A. (1991). Crop improvement for temperate australia - future opportunities. *Field Crops Research* 26(2): 141-169.

Sadras, V. O. & Denison, R. F. (2009). Do plant parts compete for resources? An evolutionary viewpoint. *New Phytologist* 183(3): 565-574.

Sadras, V. O. & Richards, R. A. (2014). Improvement of crop yield in dry environments: benchmarks, levels of organisation and the role of nitrogen. *Journal of Experimental Botany* 65(8): 1981-1995.

Sadras, V. O. & Slafer, G. A. (2012). Environmental modulation of yield components in cereals: Heritabilities reveal a hierarchy of phenotypic plasticities. *Field Crops Research* 127(0): 215-224.

Shepherd, K., Cooper, P., Allan, A., Drennan, D. & Keatinge, J. (1987). Growth, water use and yield of barley in Mediterranean-type environments. *The Journal of Agricultural Science* 108(2): 365-378.

Slafer, G. A., Araus, J. L., Royo, C. & Del Moral, L. F. G. (2005). Promising eco-physiological traits for genetic improvement of cereal yields in Mediterranean environments. *Annals of Applied Biology* 146(1): 61-70.

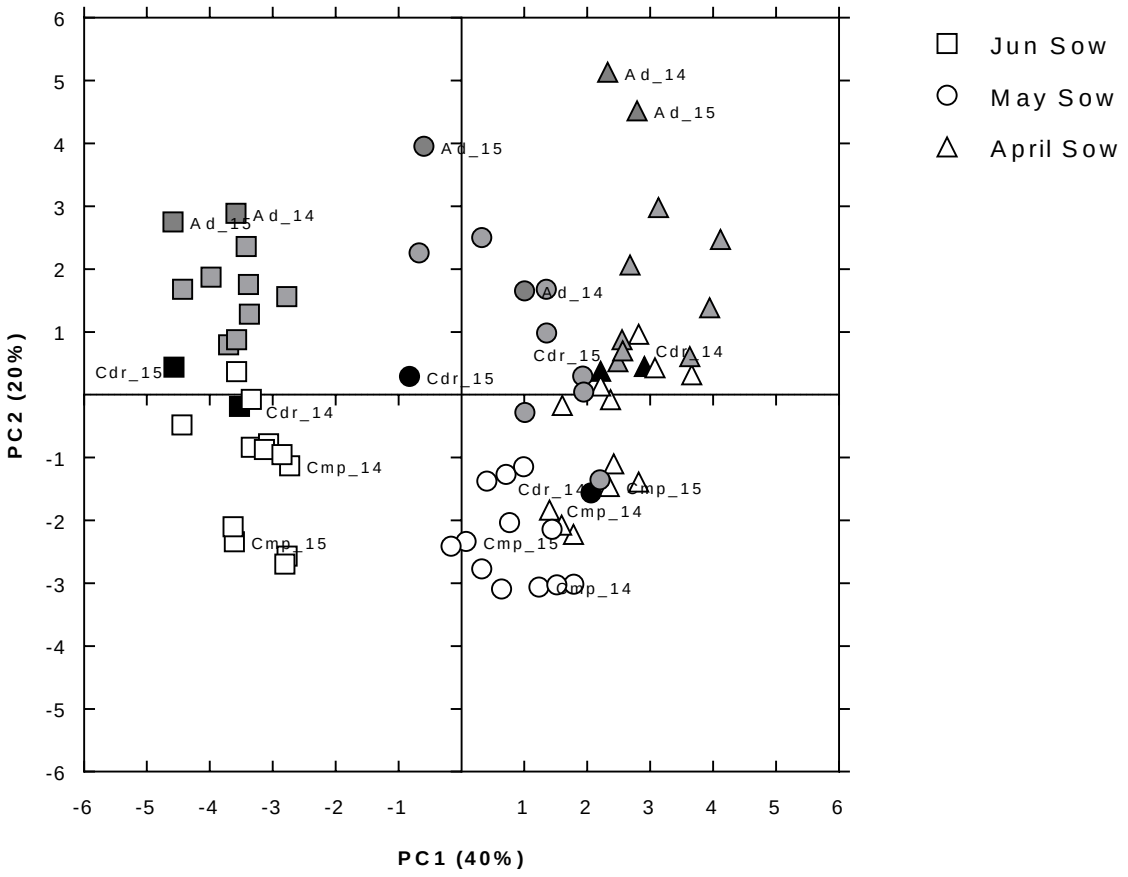
Slafer, G. A. & Rawson, H. M. (1994). Sensitivity of wheat phasic development to major environmental-factors - a reexamination of some assumptions made by physiologists and modelers. *Australian Journal of Plant Physiology* 21(4): 393-426.

Slafer, G. A., Savin, R. & Sadras, V. O. (2014). Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research* 157(0): 71-83.

Waddington, S. R., Cartwright, P. M. & Wall, P. C. (1983). A quantitative scale of spike initial and pistil development in barley and wheat. *Annals of Botany* 51(1): 119-130.

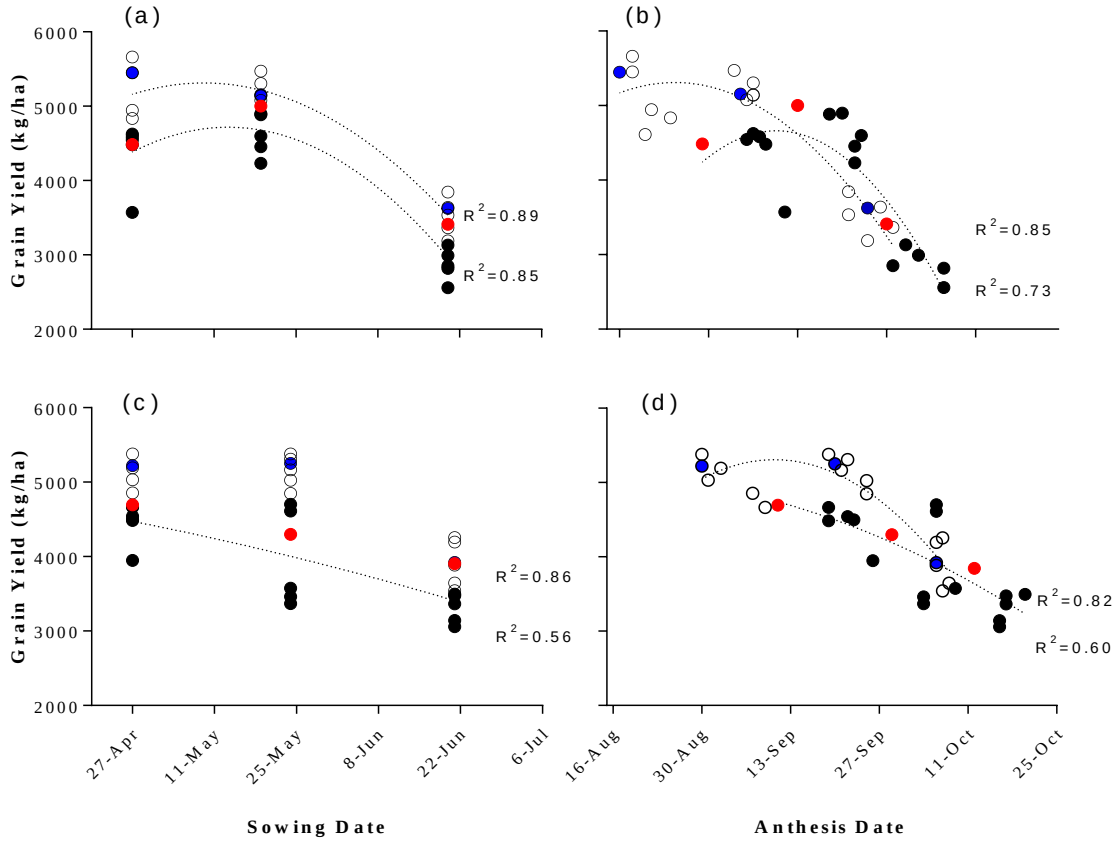
Zadoks, J. C., Chang, T. T. & Konzak, C. F. (1974). A decimal code for the growth stages of cereals. *Weed research* 14(6): 415-421.

Supplementary tables and figures:



Supplementary Figure 3 Scores plot of the principal components analysis considering the yields of the 12 genotypes in all six growing environments Δ= April, ○ May, □ = June sow.

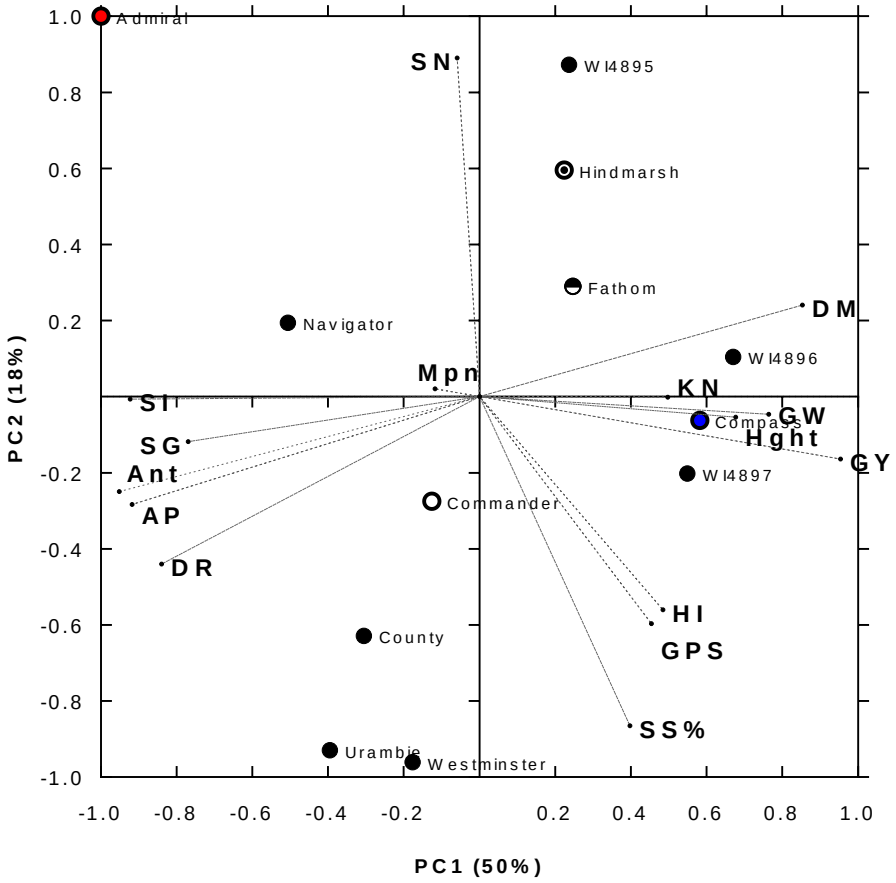
Ad= Admiral, Cmp = Compass, Cdr = Commander in the season _14 and _15 (2014 and 2015). Shaded symbols are slower developing lines and open symbols faster developing.



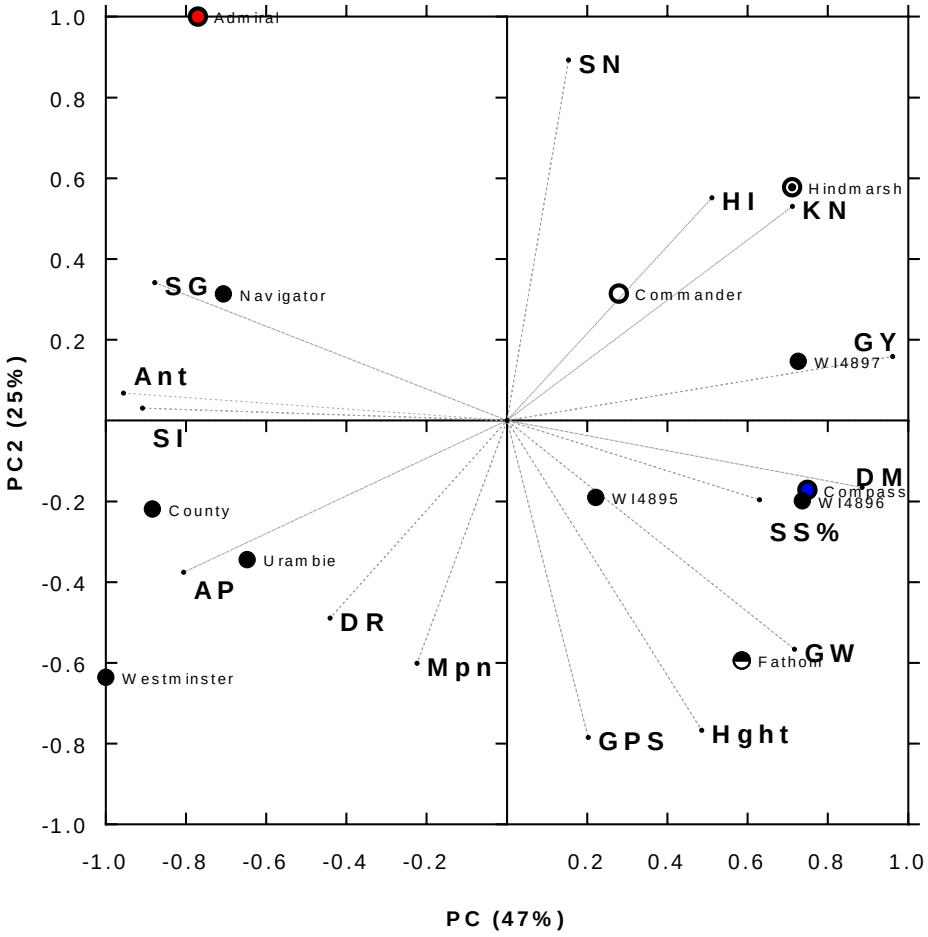
Supplementary Figure 4 Relationship between grain yield and sowing date in 2014 (a), and 2015 (c). Relationship between anthesis date and grain yield in 2014 (b), and 2015 (c). Slow development group (closed symbols), fast development group (open symbols). Compass (blue symbols), Commander (red symbols)

Supplementary Table 4. Pearson correlations between development, yield and its components Dry Matter (DM), Spike per m2 (SN), Grain Number (KN), Grain Weight (GW), Grain Yield (GY), Height (Hght), Spikelet Survival (SS%), Grains per spike (GPS), Time to Double Ridge (DR), Time to Awn Primordia (AP), Time to Anthesis (Ant), Duration Spike Initiation (SI), Duration of critical phase (SG). Data are relative to the mean (1.0) of each experiment and include both genotypic and environmental factors. Statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and NS ($P > 0.05$).

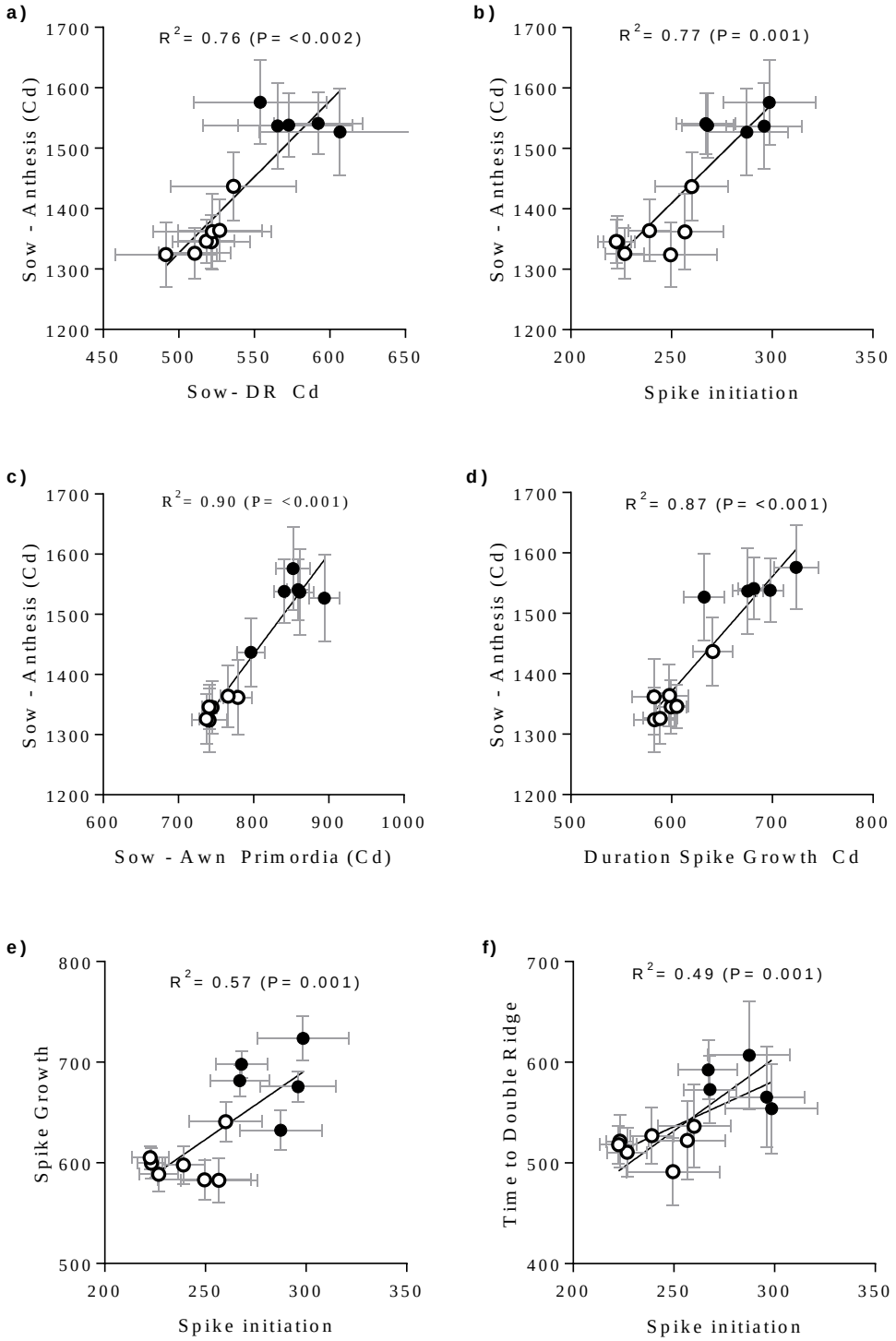
	Time to Anthesis	Time to Double Ridge	Duration Spike Initiation	Duration of critical phase	Time to Awn Primordia	Max Primordia Number	Spikelet Survival	Grains per spike	Grain Weight	Spike per m2	Grain Number	Dry Matter	Height	Harvest Index		
Time to Double Ridge	0.75***															r
Duration Spike Initiation	0.85***	0.52***														*1.0*
Duration of critical phase	0.86***	0.39**	0.67***													*0.8*
Time to Awn Primordia	0.9***	0.91***	0.83***	0.58***												*0.6*
Max Primordia Number	0.21ns	0.18ns	0.32**	0.08ns	0.28*											*0.4*
Spikelet Survival	-0.36*	-0.11ns	-0.46***	-0.33*	-0.30*	-0.37*										*0.2*
Grains per spike	-0.2ns	0.02ns	-0.23ns	-0.26*	-0.09ns	0.3*	0.77***									*0.0*
Grain Weight	-0.62***	-0.3*	-0.63***	-0.6***	-0.5***	0.09ns	0.25*	0.29*								*-0.2*
Spike per m2	-0.09ns	-0.27*	0.03ns	0.01ns	-0.17ns	-0.41**	-0.46***	-0.74***	-0.36**							*-0.4*
Grain Number	-0.39**	-0.37**	-0.25*	-0.33**	-0.37**	-0.31**	0.3*	0.1ns	-0.23ns	0.53***						*-0.6*
Dry Matter	-0.71***	-0.44**	-0.63***	-0.67***	-0.6***	-0.13ns	0.23ns	0.12ns	0.65***	0.2ns	0.42**					*-0.8*
Height	-0.48***	-0.17ns	-0.5***	-0.5***	-0.35**	0.18ns	0.32**	0.43**	0.64***	-0.33*	0ns	0.58***				*-1.0*
Harvest Index	-0.39**	-0.28*	-0.33**	-0.35**	-0.35**	-0.29*	0.63***	0.45**	-0.05ns	-0.08ns	0.57***	0.01ns	-0.11ns			
Grain Yield	-0.79***	-0.52***	-0.71***	-0.73***	-0.69***	-0.29*	0.58***	0.38**	0.46***	0.10ns	0.68***	0.77***	0.38**	0.64***		



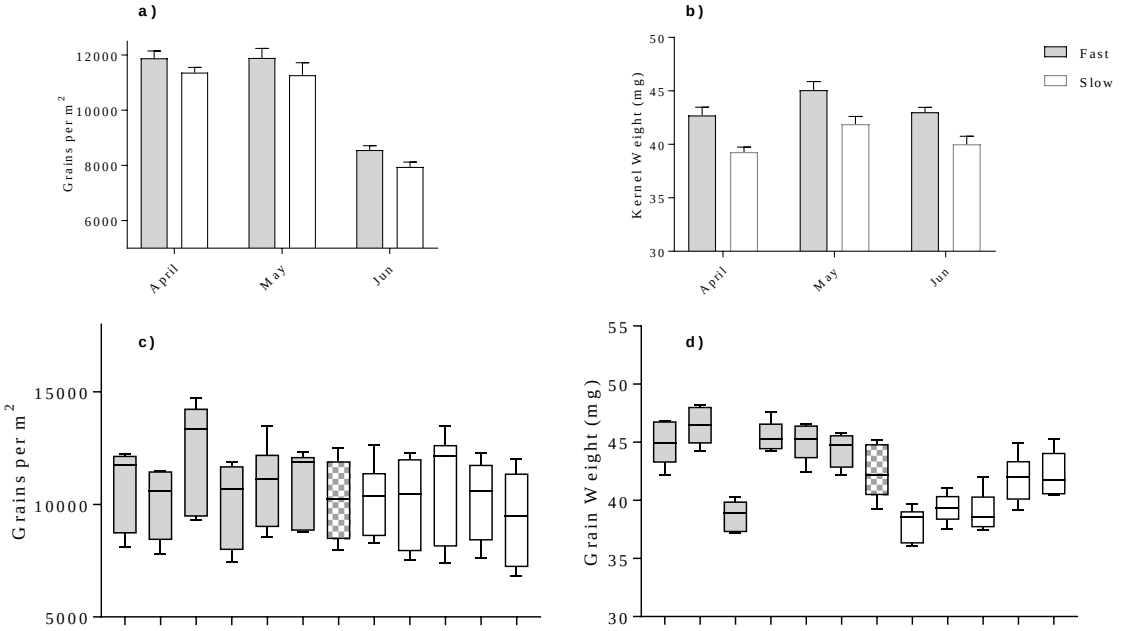
Supplementary Figure 5 Biplot combining the PCA scores and loadings for April sowing genotypic variability prevailing in the 12 genotypes in terms of Dry Matter (DM), Spike per m2 (SN), Grain Number (KN), Grain Weight (GW), Grain Yield (GY), Height (Hght), Spikelet Survival (SS%), Grains per spike (GPS), Time to Double Ridge (DR), Time to Awn primordia (AP), Time to Anthesis (Ant), Duration Spike Initiation (SI), Duration of critical phase (SG)



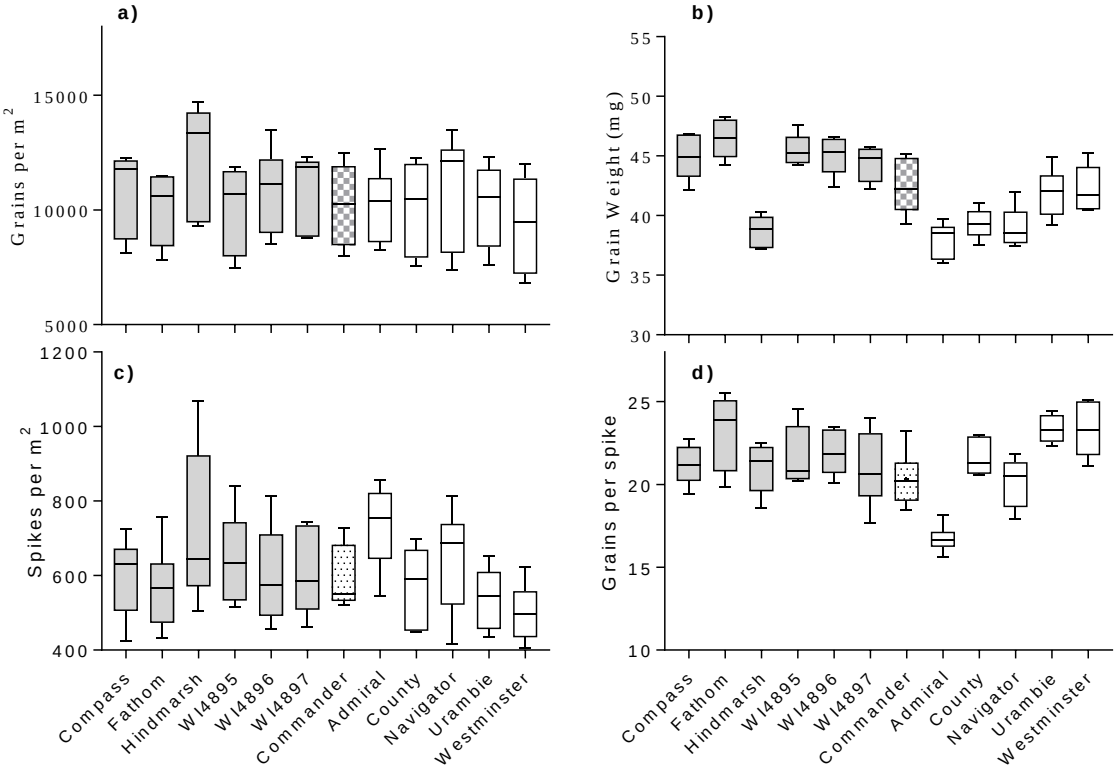
Supplementary Figure 6 Biplot combining the PCA scores and Loadings for June sowing genotypic variability prevailing in the 12 genotypes in terms of Dry Matter (DM), Spike per m2 (SN), Grain Number (KN), Grain Weight (GW), Grain Yield (GY), Height (Hght), Spikelet Survival (SS%), Grains per spike (GPS), Time to Double Ridge (DR), Time to Awn Primordia (AP), Time to Anthesis (Ant), Duration Spike Initiation (SI), Duration of critical phase (SG)



Supplementary Figure 7. Relationships between thermal time to anthesis and either sowing to awn primordia (a), double ridge (b), and relationship between these two component phases. Each data-point is the average across the six experiments and error bars are the standard errors of the means (not seen when smaller than the size of the symbol). Open circles represent the fast and closed circles represent the slow developing lines.



Supplementary Figure 8 The effect of sowing data on (a) average grains per m^2, and (b) kernel weight and boxplots showing (c) grains per m^2, and (d) thousand grain weight among varieties for the six field environments. Shaded boxes and bars indicate the fast developing genotypes, and unshaded the slow developmental group of genotypes.



Supplementary Figure 9 Average spikes per m2 across April, May, and June sowing environments, and boxplot (b) across genotypes in all six environments. Bar graph for (c) average grains per spike per m2 across April, May, and June sowing environments, and boxplot (d) across genotypes in all six environments. Shaded boxes and bars indicate the fast developing genotypes, and unshaded the slow developmental group of genotyp

CHAPTER 5: Genetic analysis of yield and adaptation in the narrow bi-parental barley population Commander x Compass.

Statement of Authorship

Title of Paper	Genetic analysis of yield and adaptation in the narrow bi-parental barley population Commander x Compass.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	

Principal Author

Name of Principal Author (Candidate)	Kenton Porker
Contribution to the Paper	Developed population, conducted phenotyping and analysis on all samples, interpreted data, wrote manuscript, edited manuscript and acted as corresponding author.
Overall percentage (%)	75%
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: 26 February 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Timothy March
Contribution to the Paper	Conducted genotyping and developed genetic map
Signature	Date: 27 Feb 2018.

Name of Co-Author	Glenn McDonald
Contribution to the Paper	Assisted with data interpretation, Editing and reviewing manuscript
Signature	Date: 27/2/18

Name of Co-Author	Neil Fettell		
Contribution to the Paper	Idea development, experimental design, editing and reviewing manuscript		
Signature		Date	27 February 2018

Name of Co-Author	Jason Eglinton		
Contribution to the Paper	Idea development, development of germplasm, editing and reviewing manuscript		
Signature		Date	28TH FEBRUARY 2018

Name of Co-Author	Stewart Coventry		
Contribution to the Paper	Idea development, development of germplasm, data interpretation editing and reviewing manuscript		
Signature		Date	28TH FEBUARY 2018

Please cut and paste additional co-author panels here as required.

Genetic analysis of yield and adaptation in the narrow bi-parental barley population Commander x Compass.

Porker KD[1]., Coventry S[1]., March T[1]., Fettell NA[2]., McDonald GK[1] & Eglinton JK[3]

1 School of Agriculture, Food and Wine, Faculty of Sciences, The University of Adelaide, Waite Campus, PMB 1, Glen Osmond SA, Adelaide 5064, Australia

2 University of New England and Central West Farming Systems, Condobolin, NSW, 2877, Australia

3 Sugar Research Australia, Bruce Highway, Gordonvale, Qld 4865, Australia

Abstract:

Genetic improvement in yield potential is a primary objective of barley breeding programs. The recently released variety Compass represents a step change in yield potential, showing a consistent yield improvement over current varieties across different environments. The objective of this study was to identify the genetic basis of crop development and adaptation contributing to improved yield and kernel weight of Compass compared to the current malting benchmark Commander. A bi-parental Compass x Commander population was developed and planted in field trials at three sowing times at Roseworthy, South Australia to determine the quantitative trait loci (QTLs) controlling development, and their association with agronomic traits related to grain yield. Across all sowing times, Compass showed on average a 6% higher kernel weight compared to Commander, accompanied by more grains per spike with later sowing. Compass showed a different sensitivity to photoperiod than Commander. It developed faster than Commander did under the short photoperiods associated with early May sowing dates, similarly to Compass at a June sowing, but slower when grown over summer. Development was predominantly associated with QTL near the photoperiod response gene (*Ppd-H1*) on chromosome 2H. This is a new finding as it is a shift away from the photoperiod responsive cultivars previously considered a requirement for adaptation to Australian environments. In addition this study detected QTL that infers the effects of alleles from Compass on chromosome 6H that contributed to fast development under the short photoperiods

associated with May sowing, and other QTLs on 3H and 5H irrespective of photoperiod and temperature environments. There were many pleiotropic effects of the loci near *Ppd-H1*, however two major kernel weight QTL on 4H and 6H, and spike length on 6H were detected that were independent of developmental QTL. This demonstrated it is possible to improve kernel weight and spike length within cultivars with similar heading times but differing photoperiod sensitivity. Furthermore QTL for canopy architecture and NDVI were co-located with kernel weight and spike length QTL, suggesting that greater kernel weight and/or grains per spike in Compass was likely due to improved synchronisation of growth with development leading to optimal resource allocation to the developing inflorescences prior to and/or post anthesis. The QTL effects detected are relevant to the control of yield and adaption related to conventional sowing dates and commercial farming environments in southern Australia. The developmental QTL identified in this study provides scope to further fine tune development and yield components under very small changes in photoperiod associated with early May – Jun sowing dates. Our results also highlight that breeders should consider selecting for a diverse range of phenology types in summer nurseries and it is possible to improve yield and kernel weight in lines with photoperiod insensitivity.

Introduction:

Breeding for stable high yield in Mediterranean environments is difficult and slow due to variability in climatic stresses (Baum et al., 2003; Mansour et al., 2014). Crop development is the main factor driving yield and adaptation (Richards, 1991) and plant breeders have successfully improved yield within varieties that flower within a narrow range known as the "optimum flowering window" (Young and Elliott, 1994) to reduce exposure to frost, heat and water stress at sensitive periods of crop development. Commander barley is the current benchmark malting variety in South Australia but a recent release, Compass, has consistently out-yielded Commander by 10% via improvements in grain number per spike and heavier kernel weight (Chapter 4). This is a step change by commercial standards. The genetic link between crop development and yield adaptation in stable, high yielding varieties such as Compass has not been identified. Compass was derived from the European cultivar County, backcrossed to Commander, and is therefore genetically similar to Commander. Finding any developmental variability in a Compass x Commander mapping population will be of importance for understanding adaptation to Mediterranean environments like southern Australia.

Pleiotropic effects of developmental regulators and the coordination of growth with flowering may be part of a reproductive strategy to optimize resource allocation to the developing inflorescences and seeds leading to improved kernel weight and or more grains per spike (Digel et al., 2016). The main factors controlling development in barley are photoperiod response (Roberts et al., 1988) vernalisation response (Fu et al., 2005) and earliness *per se* (Gallagher et al., 1991). Ultimately the pattern of development reflects how these development controls interact with the environment. Barley is a long-day plant that flowers earlier as photoperiod increases (Laurie et al., 1994). Photoperiod sensitivity, minimal vernalisation responses and a short basic vegetative phase (BVP) has long been regarded as a requirement for adaptation to Australian conditions. Australian breeders have targeted a plant type with a short basic vegetative phase (BVP) (Major, 1980) by selecting genotypes with a short duration to flowering under long days in summer nurseries leading to enrichment of photoperiod sensitivity alleles (Boyd et al., 2003).

The major photoperiod-sensitive genes are located at two *Ppd* (photoperiod) loci, *Ppd-H1* and *Ppd-H2*. The *Ppd-H1* locus is located on the short arm of chromosome arm 2H, and is a principal inducer of flowering under long days In barley (Laurie et al., 1994; Börner et al., 2002; Karsai et al., 2008; Ren et al., 2010) while *Ppd-H2* is on 1HL and influences flowering under short days (Laurie et al., 1995; Faure et al., 2012). *Ppd*- H1 is a pseudo-response regulator gene (HvPRR37), the recessive allele *ppd*-H1 is the major causes of the reduction in photoperiod response in European spring types and hence the reason for late flowering at long photoperiods (Turner et al., 2005; Alqudah et al., 2014) .

Many QTL for heading date are often linked with yield in barley (Bezant et al., 1996; Rollins et al., 2013) and associated with the major phenology genes such as *Ppd*-H1 , *Ppd*-H2 and vernalisation requirement genes (*Vrn*-H1, *Vrn*-H2, and *Vrn*-H3) (Cockram et al., 2007), and the EPS2 locus (Laurie et al., 1994; Tondelli et al., 2014) generally reflects the BVP. Additional to yield many Australian mapping populations have identified genomic regions affecting kernel weight, with most QTL also associated with plant development, mainly *Ppd*-H1, *EPS*2, and well as the semi-dwarfing gene Denso (*Sdw1*) (Coventry et al., 2003). It is therefore feasible to expect links between developmental responses, biomass accumulation and resource

allocation to yield components. Many authors suggest improvement of yield in Mediterranean conditions may come through direct selection for a combination of more stable QTL involved in the expression of traits significantly correlated with yield (Teulat et al., 2001).

Developmental patterns strongly influence grain yield formation, particularly as the period of growth from awn primordia to tipping has been suggested to be the most critical phase for determining grain number (Alqudah and Schnurbusch, 2014). Extending the length of the phase with differing phenology genes may hold promise for improving yield (Alqudah and Schnurbusch, 2014). The other component of yield, kernel weight, is influenced by developmental traits that affect assimilate accumulation prior to anthesis and supply to the developing grain post anthesis, as well as being directly affected by the timing of flowering due to exposure solar radiation, temperature and moisture availability for grain fill (Coventry et al., 2003). For example, senescence is recognised as an adaptive strategy used by plants to respond to environmental cues such as changes in photoperiod. Maintenance of green colour during grain filling (stay green) has been proposed as an important trait for improved grain plumpness by prolonging photosynthesis (Thomas et al., 2014).

Yield and phenology studies often use populations based on diverse parents causing wide segregation for flowering date. However, in Australian environments flowering must occur within a narrow range and extreme phenology responses can become a confounding factor leading to large QTL × environment (QTL x E) interactions (Romagosa et al., 1999) for grain yield. Therefore, finding stable QTL for high yield is difficult. To achieve genetic gain in yield potential and adaptation to Australian environments it will be important to identify developmental loci responsible for the determination of grain number (Araus et al., 2008) and kernel weight within elite cultivars of similar flowering time, with high heritability and limited environmental interaction.

The other factor often overlooked in development genetic studies is sowing date. Sowing date is a management option utilised by growers to synchronise crop development to environment. In southern Australia, barley has typically been sown in mid-May to early June; however, over the last decade there has been a trend towards earlier May sowing to maximise yield potential. Changes in sowing date expose genotypes to different photoperiod and temperature regimes

and therefore earlier sowing may require a different pattern of development so that flowering occurs at a time when the risk of frost and drought are low. A recent study conducted by Obsa et al. (2016) of elite crosses including Commander found none of the major developmental genes, including *Ppd-H1*, *Vrn-H1*, *Vrn-H2* and *Vrn-H3*, significantly influenced yield. This suggests there maybe yield QTL independent of flowering time. However, it is important to note that the planting dates ranged from May 20 – June 27, slightly later than conventional commercial sowing times in their study. To the best of our knowledge, there are no published studies on the genetic basis of phenology in adapted lines within the range of sowing dates over which barley is currently sown in the medium rainfall region of South Australia.

The objective of this study was to identify the genetic basis of improved yield, kernel weight and adaptation of Compass compared to Commander barley by developing a bi-parental mapping population and exposing the population to three sowing times. The major discussion for this paper will focus on the key loci controlling crop phenology across three sowing environments in Compass barley.

Materials and Methods:

Plant Material

A population of 1200 F2:5 recombinant inbred lines was derived from the Compass x Commander pair-wise reciprocal cross. A subset of 601 RILs was chosen for genotyping and phenotyping based on a combination of seed availability, cost of genotyping and the resources available for phenotyping.

Compass and Commander are Australian two-rowed malting varieties developed by the University of Adelaide. Commander (Keel/Sloop//Galaxy) is a malting variety representing an established benchmark for grain yield and grain size in medium rainfall environments of Australia (www.nvtonline.com.au). Compass (WI3416/County//Commander) was derived by a cross between Commander and the European cultivar, County. Commander is a pure seed reselection from WI3416, so essentially Compass was derived from a backcross to Commander. Commander and Compass were granted variety registration in 2005 and 2014 respectively (www.ipaustralia.com.au).

Statistical design and analysis of field trials

The parents and the 601 population lines were planted in un-replicated 1 metre rows in a summer nursery located at Virginia (34°38′S, 138°35′E) in South Australia sown on the 23rd December 2015. Yield plots were sown over three sowing dates at Roseworthy in 2016. Different numbers of lines were planted in partial replicated yield trials at three sowing dates: May 5 (ENV1), May 20 (ENV2) and June 15 (ENV3). ENV1 and ENV2 contained 370 genotypes and ENV3 contained 173 (Supplementary Table 5). Planting dates were chosen to expose genotypes to different photoperiod and temperature regimes relevant to commercial practice in the medium rainfall region of South Australia. Rainfall, temperature, and daylength statistics are presented in Table 13. Day lengths, including civil twilight were calculated using the formulae of Forsythe et al. (1995). Thermal time (°CxD, GDD) was calculated as the mean of the daily maximum and minimum air temperature using 0 °C as a base temperature.

Yield plots had a density of 150 seeds/m^2 planted in five rows (21.5 cm row spacing) by 3 m long. Experiments received 15 kg P/ha as diammonium phosphate at sowing, with 46 kg N ha^{-1} (as urea - 46% N) top-dressed during mid-tillering. Weed management and disease control followed normal commercial practice using herbicides and fungicides at recommended rates. Growth and yield of the plots were not adversely affected by disease or weed competition.

Table 13. Monthly and long-term weather statistics for the growing season at Roseworthy, SA

	April	May	June	July	Aug	Sep	Oct	Nov	Mean (Apr - Nov)	Total Sum (Apr - Nov)
						Maximum temperature (°C)				
Mean	24	19.8	16.2	15.3	16.5	19.8	23.6	27.6	20.35	
2016	25.5	20.4	15.8	15	17	16.8	20	25.8	19.5	
						Minimum temperature (°C)				
Mean	10.4	8.7	6.5	5.8	5.2	6.5	7.6	10.6	7.6	
2016	10.7	10.8	7.9	6.6	5.4	6.5	7.4	7.9	7.9	
						Rainfall (mm)				
Mean	36.6	47.9	53.9	51.5	53.6	48.4	42.2	27.9		362
2016	10	87.4	71.4	71	52.8	108.4	54	17.4		472
						Photoperiod (hrs)				
Mean	11.06	10.15	9.69	9.91	10.70	11.74	12.84	13.80		

Phenotyping:

Phenotypic data were collected in each sowing time for grain yield (GY, t ha^{-1}), measured as the weight of grain combine-harvested per plot. Kernel weight (KW, mg) estimated from a sample of 250 grains and number of grains per unit area (GN, m^{-2}) was calculated by dividing grain yield by kernel weight. Days to awn appearance (DAA) was recorded as the number of days from sowing to when 1 cm of awns were visible on 50% of the stems in each plot. This is considered an appropriate surrogate for flowering time in this study as previous experience has shown that flowering consistently occurs within the range of 1 – 3cm awn emergence across April – June sowing times in Compass and Commander. Maturity was scored in the summer nursery using the Zadoks scale (Zadoks et al., 1974) at a single time point when the population average growth stage was at anthesis. Growing degree days to awn appearance (GDDAA) was determined using the sum of thermal time from sowing to awn appearance.

The normalized difference vegetation index (NDVI) within each time of sowing was measured on a plot basis a number of times to coincide with mean growth stages tillering (Z22), flag leaf sheath opening (Z47), flowering (Z65), and grain fill (Z75) to assess variation in greenness and leaf area index using a digital RapidSCAN CS-45 Handheld Crop Sensor (Holland Scientific).

Canopy growth habit at flag leaf emergence was scored based on a visual assessment scale from 0-5 (0 = erect and open canopy, 5 = prostrate growth habit) (Figure 14).

Figure 14. Example of canopy habit score of 1 (on the left) and a more prostrate canopy with a score of 4 (on the right) in the Compass x Commander mapping population.

Apical Dissection:

All genotypes in ENV3 and a sub selection of 55 lines that were connected across all three sowing environments were chosen randomly for apical dissection. Five plants were collected from each plot and prepared for dissection. The stages of apical development were quantified using the developmental scale of Waddington et al. (1983). The nomenclature 'W' developmental score followed by the number according to the Waddington scale was used to quantify inflorescence development. Floral development included stages from W3 (glume primordium visible) to W10 (style and stigmatic branches spreading and green anthers visible). The apical dissection for the subset of lines in ENV1 and ENV2 and all lines in ENV3 using the Waddington development scale was targeted when Compass reached W4 (awn primordia).

Spike Length and grains per spike:

Within the subset of 55 lines across all three sowing environments, ten randomly selected plants were harvested at physiological maturity and the length and grains per spike were measured using a ruler and by counting fertile spikelets on the main culm. In ENV2 and ENV3, a further five randomly selected main spike heads were collected from every plot to quantify spike length.

Genotyping:

Genomic DNA was extracted from leaf samples obtained from seedlings. Genotyping by sequencing was performed as described by (Poland et al., 2012). Genomic DNA (200 ng) of individual lines were double digested with PstI–MspI. All individuals were then ligated with unique barcoded adapters and combined into 96-plex pools. Each pool was sequenced on a single lane of an Illumina HiSeq 2000 at the Australian Genome Research Facility Ltd (Australia). Bi-allelic SNP markers were called using the Tassel UNEAK pipeline (Lu et al., 2013). Markers with greater than 20% missing data were removed. The marker sequences were aligned to the barley reference genome sequence RefSeq v1.0 (Mascher et al., 2017) using blastn (Altschul et al., 1990).

Statistical and QTL mapping analyses

Adjusted means were obtained in GenStat 18th edition using spatially adjusted REML mixed models to obtain the best linear unbiased estimates (BLUEs); spatial adjustment was included in the analysis by adding the effect of row and column according to the location of each plot in the field. Pearson's phenotypic correlations were calculated from the adjusted means. The QTL mapping analyses were performed in GenStat using a single marker regression, and the threshold used to adjust for multiple comparisons conducted using the method of (Li and Ji, 2005) with an overall significance level of 0.05. QTL mapping was performed for each trait combined across environments, and by single trait within an environment. Single site QTL analysis for Waddington development was conducted in ENV3. Spike length QTL analysis was conducted in ENV2 and ENV3.

Due to limited dissections, the subset data was used to test effect of the DAA and Spike length QTL on inflorescence development and spike length analysis of variance using the markers closest to the QTLs and environment as factors in GenStat 18. Further potential interactions between pairs of QTLs for grain yield, kernel weight, and days to awn appearance, were analysed using an unbalanced analysis of variance using the two markers closest to the QTLs and environment as factors. Principal component analysis (PCA) was performed with the Unscrambler v 10.3 software (Camo Norway) to examine genotype x trait relationships within each sowing environment. BLUE trait values were used and the data was standardised based on the mean and standard deviation. The results of the PCA are shown as bi-plots of PC1 vs. PC2 for each sowing date environment, where genotypes and traits are represented by markers on the bi-plot.

Results:

Environmental conditions

The three sowing dates and the summer nursery exposed genotypes to a range of environmental conditions including different temperature and photoperiod regimes (Table 14). At Roseworthy, the differences in mean photoperiod during the period from sowing to W4 were small and lowest in ENV2. The mean duration to awn appearance for the population was 109 days in ENV1, 109 days in ENV2, and 99 days in ENV3. The mean photoperiod from sowing to anthesis increased from 10 h at ENV1 to 10.5 h at ENV3. Temperatures declined from ENV1 to ENV3. The rainfall for 2016 was significantly greater than the long-term average for Roseworthy and temperatures were generally milder than average (Table 13), which resulted in large amounts of vegetative growth, relatively little heat and water stress during spring and high yields.

Table 14. Mean temperature and photoperiods and growing degree days (GDD) for each environment (ENV1-3) during the period from sowing until when the apical dissection for Compass was conducted (W4) and time from sowing to a mean population Zadoks score of 65 (Z65).

	Summer Nursey	ENV1		ENV2		ENV3	
	Z65	W4	Z65	W4	Z65	W4	Z65
Photoperiod (hrs)	14.0	9.9	10.0	9.8	10.2	10.0	10.5
Min Temp	16.9	9.3	7.9	7.7	7.0	6.8	6.5
Max Temp	31.5	18.0	16.9	16.2	16.3	15.2	16.1
Avg Temp	24.3	13.6	12.4	11.9	11.7	11.0	11.3
GDD	910	723	1352	751	1272	671	1104

Variation in yield, kernel weight and phenology

Yield differences between the parents were observed at each time of sowing date (Table 15). Commander yielded 0.62 t/ha more than Compass at the earliest sowing date, whereas at the other two sowing dates Compass out yielded Commander by 0.39 t/ha and 0.31 t/ha respectively. The population showed transgressive segregation for yield at all three times of sowing (Supplementary Figure 10). The heritability for yield ranged from 0.21 to 0.29. ENV1 was the lowest yielding environment with a mean yield of 7.38 t/ha due largely to high amount of vegetative growth and lodging, followed by 7.66 at ENV2 and 7.71 at ENV3.

Differences between the parents in kernel weight were observed at each time of sowing (Table 15). On average across all environments, Commander had a mean kernel weight of 49.5 mg and Compass 54.9 mg. The mean kernel weight of Compass was 8.9, 13.8, and 9.7 percent greater than Commander at ENV1, ENV2, and ENV3, respectively. Across environments, the mean kernel weight ranged from 50.3 mg at ENV1 to 54.1 mg at ENV3. The population showed transgressive segregation for kernel weight in all environments and the heritability ranged from 0.33 – 0.62 (Supplementary Figure 10).

Differences between parents for DAA were highest at ENV1 and ENV2. Compass reached awn appearance 8 days earlier than Commander at ENV1, and 13 at ENV2, and 3 days earlier at ENV3 (Table 15). There was noticeable transgressive segregation at each sowing date (Supplementary Figure 10) and the heritability ranged from 0.55 to 0.76.

There was variation for crop development in the summer nursery, and evidence of transgressive segregation in Zadoks growth scores over summer. The development of Compass (Z46.8) was significantly delayed compared to Commander (Z65.8), and genotypes still yet to reach growth stage 30 (Supplementary Table 8). This variation was not experienced in the autumn and winter sowing dates, when Compass was more advanced than Commander.

Table 15. Summary statistics based on BLUE s for agronomic traits grain yield, kernel weight, and days to awn peeps for the population and variation in the parents across all sowing environments.

	Grain Yield (t/ha)			Kernel Weight (mg)			Days to Awn Appearance			Grains per m^2 ($x\ 10^{-3}$)		
	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3
Compass	6.75	7.71	7.82	53.0	55.7	56.1	103.1	101.5	96.7	12.78	14.01	13.99
Commander	7.37	7.32	7.50	48.6	48.9	51.1	111.2	114.3	99.7	15.03	14.89	14.60
Mean	7.38	7.66	7.71	50.3	53.4	54.1	109.1	109.0	98.6	14.45	14.39	14.26
Min	5.72	5.78	6.29	41.2	46.0	45.5	98.5	99.2	94.5	9.25	97.66	10.98
Max	9.17	8.93	9.57	56.9	59.7	60.2	115.8	118.4	101.9	18.81	17.93	18.30
s.d.	0.57	0.56	0.57	1.9	2.4	2.3	2.8	3.6	1.3	1.30	1.10	1.10
F Pr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.01	<0.001	<0.001
Heritability	0.29	0.25	0.21	0.46	0.62	0.33	0.76	0.62	0.55	0.37	0.33	0.31

There was a weak positive association between time to awn appearance and grain yield at ENV1, but not at ENV2 and ENV3. Grain yield was predominantly explained by improved grain number at all sowing times, while kernel weight was positively associated with grain yield in ENV2 and ENV3. Time to awn appearance was strongly correlated between sowing environments ENV1 and ENV2, with the strength of the relationship decreasing with ENV3 (Table 16). Grain number was negatively correlated with kernel weight in all environments whereas time to awn appearance was negatively associated with kernel weight in ENV2 and ENV3 (Table 16, Supplementary Figure 11).

Table 16. Pearson correlation coefficient for correlations between agronomic traits Yield, Days to awn appearance (DAA), kernel weight (KW), Grains per m^2 (GN) across sowing date environments for the Compass x Commander population. Bold text corresponds to significant correlations. Statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and blank = ($P > 0.05$).

	Yield ENV1	**Yield ENV2**	**Yield ENV3**	**DAA ENV1**	**DAA ENV2**	**DAA ENV3**	**KW ENV1**	**KW ENV2**	**KW ENV3**	**GN ENV1**	**GN ENV2**
Yield ENV2	**0.22***	-									
Yield ENV3	0.01	0.09	-								
DAA ENV1	**0.21***	-0.09	-0.12	-							
DAA ENV2	**0.21***	0.00	0.03	**0.70*****	-						
DAA ENV3	0.03	0.06	-0.05	**0.34***	**0.37****	-					
KW ENV1	-0.07	**0.28***	0.10	-0.10	-0.08	**-0.29***	-				
KW ENV2	-0.08	**0.17***	0.09	**-0.35****	**-0.27***	**-0.40****	**0.41****	-			
KW ENV3	0.09	-0.03	**0.20***	0.00	-0.13	**-0.30****	0.10	**0.34****	-		
GN ENV1	**0.90*****	0.07	-0.03	**0.22***	**0.22***	0.13	**-0.50****	**-0.26***	0.03	-	
GN ENV2	**0.27***	**0.83*****	0.04	0.13	**0.15***	**0.26***	0.03	**-0.40****	**-0.20***	**0.23***	-
GN ENV3	-0.04	0.11	**0.84*****	-0.11	0.11	0.11	0.05	-0.11	**-0.37****	-0.05	0.16

Developmental QTL results:

Days to awn appearance QTL

Six QTL were identified for DAA with four QTL with LOD scores greater than 3 and two QTL less than 3 (Table 17). The largest QTL occurred on chromosome 2H (QDAA_2H) with the peak LOD score corresponding to the nearest marker TP57409. This QTL had a large effect but interacted with environment, explaining 71.7% of the total variation in ENV1, 37.4% in ENV2, and 3.9% in ENV3, with the Commander allele delaying awn appearance by 2.3 and 2.2, and 0.3 days respectively. A major QTL x E was located on 6H (QDAA_6H.2) which explained 0.7% to 5.5% of the variance respectively with the Commander allele delaying awn appearance. The Commander allele at QDAA_3H delayed awn appearance by an average of 0.3 days, and at QDAA_5H.1 the Compass allele delayed awn appearance by 0.2 days in all environments. The QTL QDAA_5H.2, and QDAA_6H.1 LOD explained more of the variation in ENV3 relative to other environments contributing a minor delay ranging from 0.3 – 0.4 days coming from the Commander allele. The QTL for growing degree days to awn appearance (GDDAA) were similar to the QTL for days (Supplementary Table 9) albeit having a slightly higher LOD score.

Photoperiod response on crop development

Given the QTL x E for days to awn appearance associated with QDAA_2H in ENV1 and ENV2, it was thought this may be due to photoperiod sensitivity. The effect of the Commander allele at QDAA_2H on delaying GDDAA was on average 58.5 °Cd in ENV1 and 60.5 °Cd in ENV2 and not significant in ENV3 when mean photoperiods were greater than 10.5. The addition of the Commander allele at QDAA_6H to genotypes with the QDAA_2H.1 Commander allele delayed GDDAA by 12.5 °Cd at ENV1 and by 35 °Cd in ENV2, and was not significant in ENV3 or genotypes with the Compass QDAA_2H.1 allele (Figure 15). The slope of the relationships represents the photoperiod sensitivity while the difference in values at the same daylength represents the effects of other developmental controls. The data suggest the Commander (Cdr) allele at QDAA_2H results in slightly greater photoperiod sensitivity.

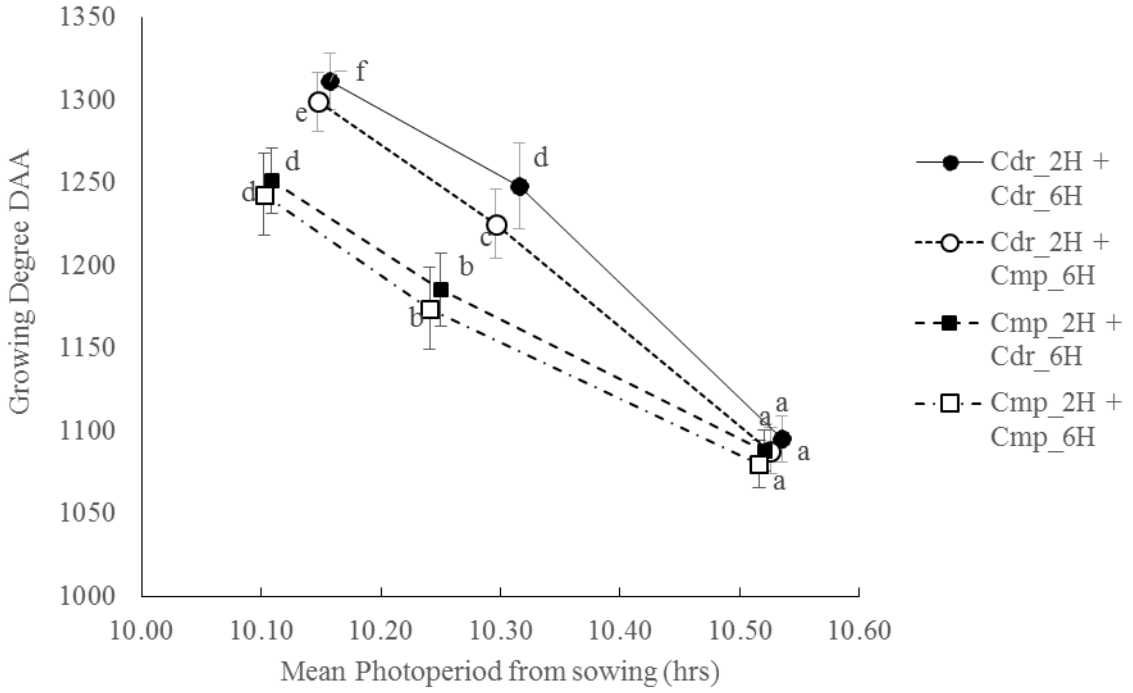


Figure 15. The association between mean photoperiod from sowing and growing degree days to awn emergence in genotypes with different allelic combinations at QDAA_2H and QDAA_6H.2. Different letters indicate significance at $P \leq 0.05$ for G x E. Bars indicate standard deviation.

Of the five QTL identified for Zadoks scores, four were coincident with those identified for DAA (Table 17). An additional QTL on 5H (QZad_5H.1) resulted in faster development from the Commander allele. The Zadoks QTL QZad_2H was at the same location as QDAA_2H and explained 92% of the phenotypic variation in Zadoks scores in the summer nursery with the

Compass allele associated with delayed development (Figure 16). The loci QZAD_3H and QZAD_5H.2 were consistent in all environments including the summer nursery suggesting constitutive expression independent of photoperiod and temperature differences.

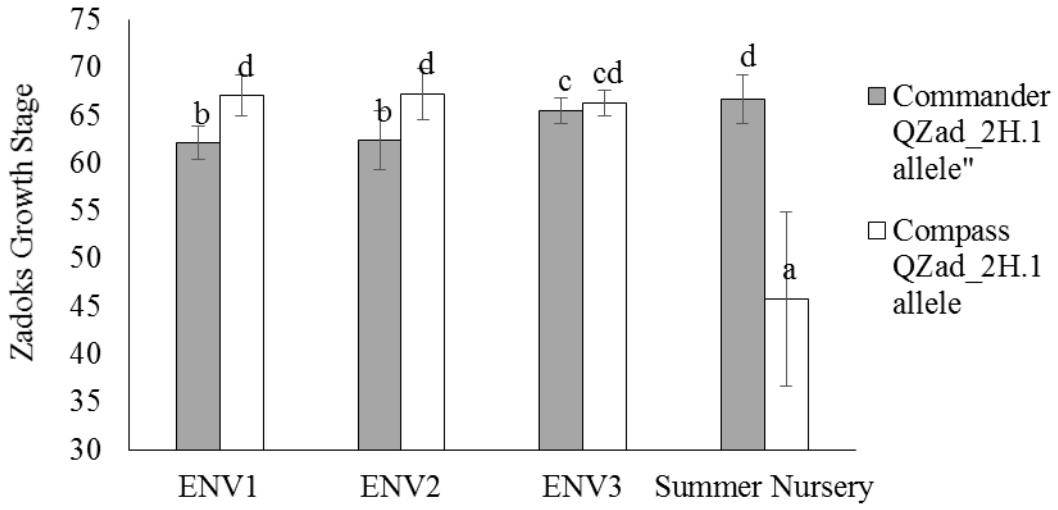


Figure 16. Mean Zadoks growth stage scores for all environments. Shaded bars represent genotypes with Commander alleles and open bars represent Compass alleles associated with the significant marker at the QZad_2H.1. The same letters are not significantly different at $P \leq 0.05$. Bars indicate standard deviation.

Inflorescence development QTL

In all environments, Compass achieved a significantly higher W score compared to Commander at the time of dissection (Figure 17

Figure 17 & Supplementary Table 6). The mean Waddington score in ENV3 for the population was W3.9, and the data ranged from W3.1 to W4.8. A single QTL was identified on the distal end of chromosome 2H in ENV3 (QWn_2H). Importantly, this was not previously detected for flowering time or any other trait (Table 17). There were not enough lines to conduct QTL analysis in ENV1 and ENV2 due to limited dissections, however QWn_2H influenced apical development in all environments, although the effect was small (0.1 – 0.2) and the Compass allele delayed development (Supplementary Table 7). However, the Commander QZad_2H allele delayed inflorescence development in the subset of lines in ENV1 and ENV2 by 0.7 and 0.4 respectively. The Commander allele at QZad_3H, and the Compass allele at QZad _5H.1 further delayed inflorescence by between 0.1 and 0.3. Despite the large effect of QDAA_2H in ENV1 and ENV2, there is evidence of variation for Waddington score in genotypes of similar duration to awn emergence (Figure 17).

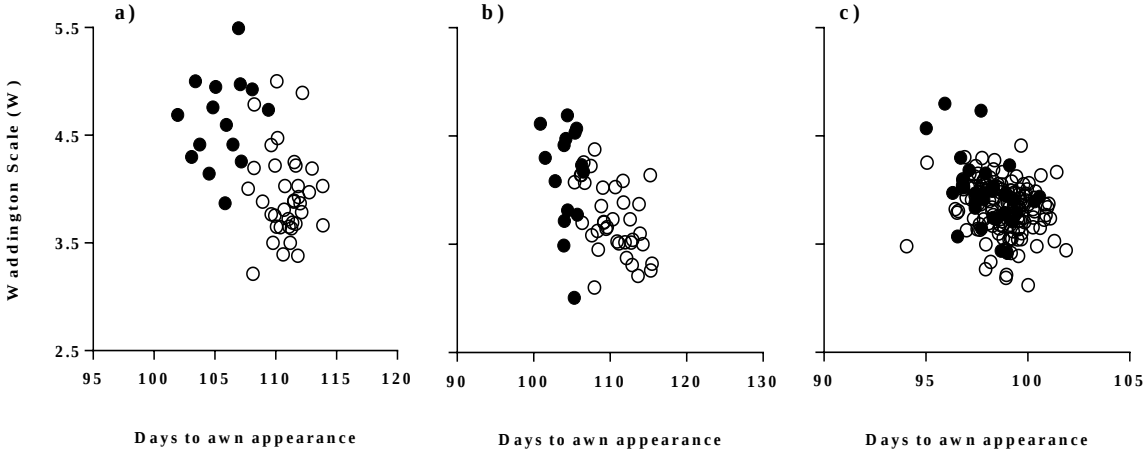


Figure 17. The relationship between Waddington development score and days to awn appearance in ENV1 (a), ENV2 (b), and ENV3 (c) with the differing alleles at QDAA_2H.1 (Commander (○), Compass (●). QDAA_2H.1 x Env (P=<0.001).

Table 17. Summary of significant developmental QTL detected for days to awn appearance (DAA), Waddington Development Score (Wn), and Zadoks, growth stage for environments 1 – 3 (E1, E2, E3) and the summer nursery (SumN)

Trait	QTL Name	Sig Marker	Chr	Base pairs	_log10 (P)	QTLxE		Additive Effect				Variance (%)				Positive allele			
							SumN	E1	E2	E3	SumN	E1	E2	E3	SumN	E1	E2	E3
DAA	QDAA_2H	TP57409	2	29202919	142.4	yes		2.3	2.2	0.3		71.7	37.4	3.9		Cdr	Cdr	Cdr
	QDAA_3H.	TP68560	3	4889	4.1	no		0.2	0.3	0.3		0.8	0.5	3.8		Cdr	Cdr	Cdr
	QDAA_5H	TP15259	5	84480924	3.8	no		0.3	0.3	0.3		0.8	0.5	3.9		Cmp	Cmp	Cmp
	QDAA_5H.1	TP85294	5	567042991	2.5	no		0.2	0.2	0.2		0.7	0.5	3.4		Cdr	Cdr	Cdr
	QDAA_6H.1	TP10034	6	9852710	2.6	no		0.2	0.2	0.2		0.6	0.3	2.7		Cdr	Cdr	Cdr
	QDAA_6H.2	TP75532	6	397073995	3.8	no		0.3	0.3	0.3		1.2	0.7	5.5		Cdr	Cdr	Cdr
Wn	QWn_2H	TP89062	2	723509052	3.6	no		na	na	0.31		na	na	10.9		na	na	Cdr
Zadoks	QZad_2H	TP72494	2	29447820	>100	yes	10.6	2.4	2.3	0.3	92	73.7	39.3	5	Cdr	Cmp	Cmp	Cmp
	QZad_3H	TP68560	3	4889	4.3	no	0.3	0.3	0.3	0.3	0.1	0.8	0.5	3.7	Cmp	Cmp	Cmp	Cmp
	QZad_5H.1	TP40204	5	219815755	4.9	no	0.3	0.3	0.3	0.3	0.1	1	0.6	4.6	Cdr	Cdr	Cdr	Cdr
	QZad_5H.2	TP85294	5	567042991	5.8	no	0.3	0.3	0.3	0.3	0.1	1.4	0.8	6.4	Cmp	Cmp	Cmp	Cmp
	QZad_6H	TP75532	6	397073995	5.0	yes	_	0.4	0.7	_	_	1.5	3.8	_	_	Cmp	Cmp	_

Yield and yield component QTL

Yield QTL

Five significant QTL were detected for yield with significant QTL x E interaction (Table 18). QYld_2H.1 on chromosome 2H was coincident with QDAA_2H.1 and QZad_2H. QYld_2H.1 had a LOD of 7.4 and explained 12.4% of the variance in ENV1 with the positive effect coming from the Commander allele but was not significant in ENV2 and ENV3. All other yield QTL had LOD scores of less than 3. QYld_2H.2 explained 8.9% in ENV2 and 27.7% in ENV3 with the positive allele coming from Compass. QYld_5H.1 had a LOD of 2.5 with the Compass allele had a large positive effect in ENV3. The QYld_5H.2 was only detected in ENV3 and explained 16.7% of the variation, with positive effect coming from the Compass allele. The final yield QTL located on 6H was only significant in ENV3 and according to the analysis explained 60% of the variation with the positive allele coming from Compass (Table 18).

Grain Number QTL

Two QTL were identified for grain number (Table 18). The QGN_2H with a peak LOD score of 10.3 was significant in ENV1 explaining 12.4% of the variance, with the positive effect coming from the Commander allele. The QTL located on 4H was relatively small and explained 1.4 – 1.8% of the variance, with the positive effect coming from the Compass allele.

Kernel Weight QTL

Six QTL were detected for kernel weight, with Compass alleles contributing to greater kernel weight in each case (Table 18). QKW_2H (coincident to QDAA_2H.1) and QKW_3H had QTL x E interaction and were only significant in ENV2 with QKW_2H adding 0.66 mg and QKW_3H adding 0.36 mg. The QKW_5H QTL was identified in ENV2 and ENV3 coincident with QDAA_5H.1. The other three QTL were stable across all three environments, with the major QTL QKW_6H having a peak LOD score of 15.9 corresponding to the nearest marker TP34779 explaining 7.7 – 12.1% of the phenotypic variation with an additive effect of 0.6 mg. QKW_4H explained 1.9 – 3.0% of the variation, adding 0.3 mg, and QKW_7H had a LOD of 7.2 explaining 2.8 – 4.3% of the variance and additive effect of 0.4 mg.

The QTL on 4H (QKW_4H) and 6H (QKW_6H) were stable across environments and appear to be independent of the major crop development QTL (QDAA_2H.1). Averaged across all environments, the Compass allele at QKW_4H and QKW_6H had an additive effect of 54.1 mg compared to 50.8 mg for the Commander allele (Figure 18). Since no epistatic interaction was detected, this increase of 6% kernel weight was independent of QDAA_2H and QZAD_2H.

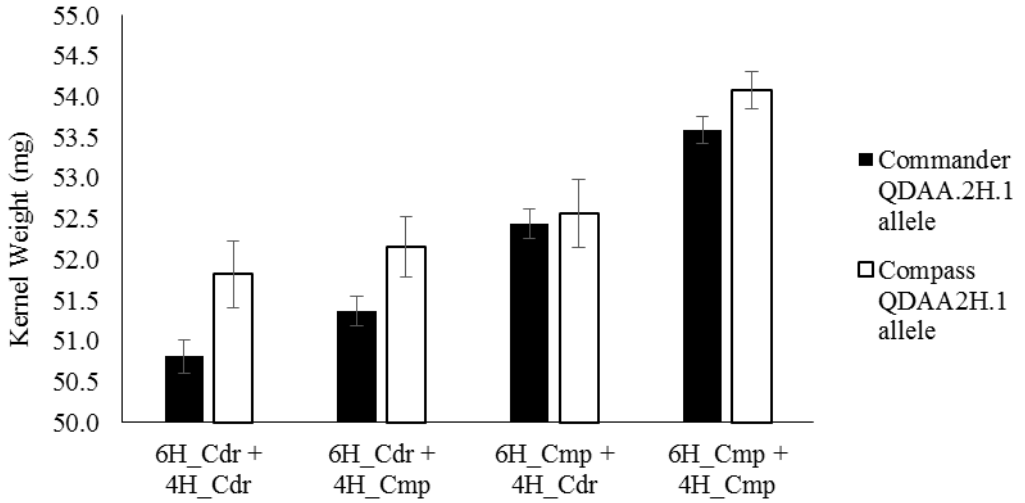


Figure 18. Mean kernel weight across all sowing dates for genotypes differing in alleles at QKW_4H, and QKW_6H. Shaded bars correspond to genotypes with the Commander QDAA_2H.1 allele, and open bars the Compass allele. Error bars are the standard error (Cdr = Commander allele; Cmp = Compass allele).

Spike Length

Length of the main culm spike ranged from 55.0 mm to 100.8 mm with transgressive segregation in all environments (Supplementary Table 6 and Supplementary Table 13). There were significant G x E interactions. Spikes of Commander were on average 8 mm longer than those of Compass in ENV1, but 8 mm and 5 mm shorter in ENV2 and ENV3 respectively. QTL analysis was conducted in ENV2 and ENV3 only. A QTL was detected on 2H (QSPL_2H) at the same location as QDAA_2H.1, however, two new QTL (QSPL_6H.1 and QSPL_6H.2) on 6H where identified and the Compass allele resulted in longer spike length (Table 18). A selection of common genotypes across environments were counted for spikelet number, and spike length was found to be highly correlated with spikelet number of the main culm in all three environments. The slope of the regressions was different for each

environment, with every 1 mm equivalent to 0.21 spikelet in ENV1, 0.28 in ENV2, and 0.22 in ENV3 (Figure 19).

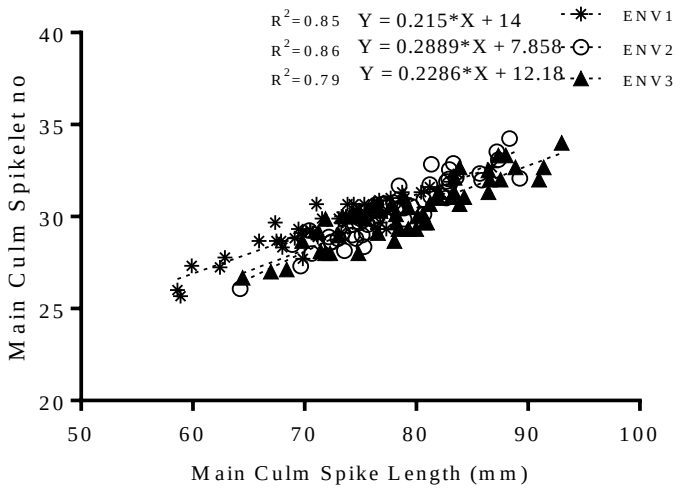


Figure 19 Relationship between main culm spike length and main culm grains per spike in a subset of lines in ENV1 (n=52), ENV2 (n=56), and ENV3 (n=57).

Due to limited genotypes in ENV 1 the ANOVA study confirmed the Compass allele at QZAD_6H.1, QSPL_6H.1, and QSPL_6H.2 lengthened spike length in all environments (Supplementary Table 7 and Figure 20). The Compass allele at QSPL_2H reduced spike length in ENV1 and ENV2, the QTLs on 6H lengthened the spike irrespective of the major development allele QZad _2H (Figure 20), and there was no significant interaction between marker pairs or environment. The QSPL_6H.1 extended spike length by 4.0, 2.5, and 6.2 mm in ENV1 – 3 respectively. According to the regressions in Figure 19 this equates to approximately one more grain per spike.

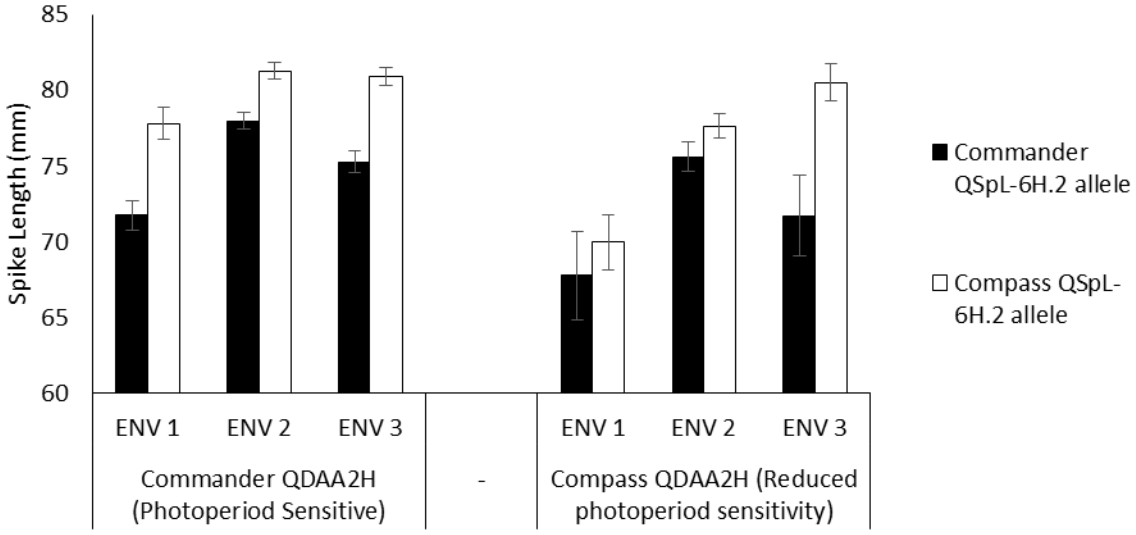


Figure 20. Mean spike length on the main culm for all environments in a) photoperiod sensitive genotypes (QZAD_2H Cdr alleles), and b) reduced photoperiod sensitive genotypes (QZAD_2H Cmp alleles). Open bars represent genotypes with Commander alleles and shaded bars represent Compass alleles associated with the significant spike length marker at the QSPl_6H.2. Bars indicate standard error.

Table 18. Summary of significant QTL detected for grain yield (Yld), kernel weight (KW), grain number (GN), and Spike Length (SpL).

Trait	QTL Name	Sig Marker	Chr	Base pairs	_log10 (P)	QTLxE	Additive Effect			Variance (%)			Positive allele		
							E1	E2	E3	E1	E2	E3	E1	E2	E3
Yld	QYld_2H.1	TP38783	2H	29454443	7.4	yes	0.19	_	_	12.4	_	_	Cdr	_	_
	QYld_2H.2	TP12549	2H	168200768	2.4	yes	0.14	0.17	0.29	_	8.9	27.7	_	Cmp	Cdr
	QYld_5H.1	TP18964	5H	45702569	2.5	yes	_	_	0.42	_	_	60.3	_	_	Cmp
	QYld_5H.2	TP65429	5H	181407038	2	yes	_	0.23	_	_	16.7	_	_	Cmp	_
	QYld_6H	TP47227	6H	225575798	2.6	yes	_	_	0.43	_	_	60.4	_	_	Cmp
KW	QKW_2H	TP14537	2H	26020946	6.0	yes	_	0.66	_	_	7.7	_	_	Cmp	_
	QKW_3H	TP82256	3H	13107711	2.6	yes	_	0.36	_	_	2.4	_	_	Cmp	_
	QKW_4H	TP53818	4H	10204053	5.3	no	0.33	0.33	0.33	3	1.9	1.9	Cmp	Cmp	Cmp
	QKW_5H.	TP85294	5H	567042991	2.4	yes	_	0.25	0.40	_	1.1	2.8	_	Cmp	Cmp
	QKW_6H	TP34779	6H	336637057	15.9	no	0.66	0.66	0.66	12.1	7.7	7.7	Cmp	Cmp	Cmp
	QKW_7H	TP41423	7H	630622135	7.2	no	0.39	0.39	0.40	4.3	2.8	2.8	Cmp	Cmp	Cmp
GN	QGNO_2H	TP38783	2H	29454443	10.3	yes	422	_	_	12.4	5.7	_	Cdr	_	_
	QGNO_4H	TP47056	4H	329469205	3.1	no	141	141	141	1.4	1.6	1.8	Cmp	Cmp	Cmp
SpL	QSPL_2H	TP57409	2H	29202919	7.1	no	na	1.45	1.45	na	6	5.9	na	Cdr	Cdr
	QSPL_6H.1	TP44838	6H	93649534	2.2	yes	na	_	1.78	na	_	8.9	na	_	Cmp
	QSPL_6H.2	TP68537	6H	180784927	3.9	no	na	1.70	1.70	na	8.2	8.1	na	Cmp	Cmp

Canopy architecture traits

There was variation for canopy structure across all environments, with Compass being more erect compared to Commander based on the visual scale (Supplementary Table 6). Within the population, scores ranged from the most erect at 0.35 to 4.41, with Commander at 2.8 in ENV1, 2.45 in ENV2, and 2.24 in ENV3. The range in canopy structure was less in ENV3 however there were fewer observations. There were six QTL for canopy structure, with the largest being both QCano2H, and QCano6H.1, with LOD scores of 45.9 and 18.0 respectively (Table 19). QCano2H.1, QCano6H.1, and QCano6H.4 were located in similar positions to major development, yield and KW QTL. All three of these QTL had the Commander allele contributing to a more closed canopy. The QCano_7H was located near to a major KW QTL (QKW_7H) which was not previously identified for development.

NDVI

Genotypic variation for NDVI across all environments depended on growth stage and time of sowing (Supplementary Table 10). There were no significant genotypic differences in all environments at Z22. Commander had a greater NDVI than Compass at Z47, Z65, and Z75, however this was more pronounced with earlier sowing (Figure 21).

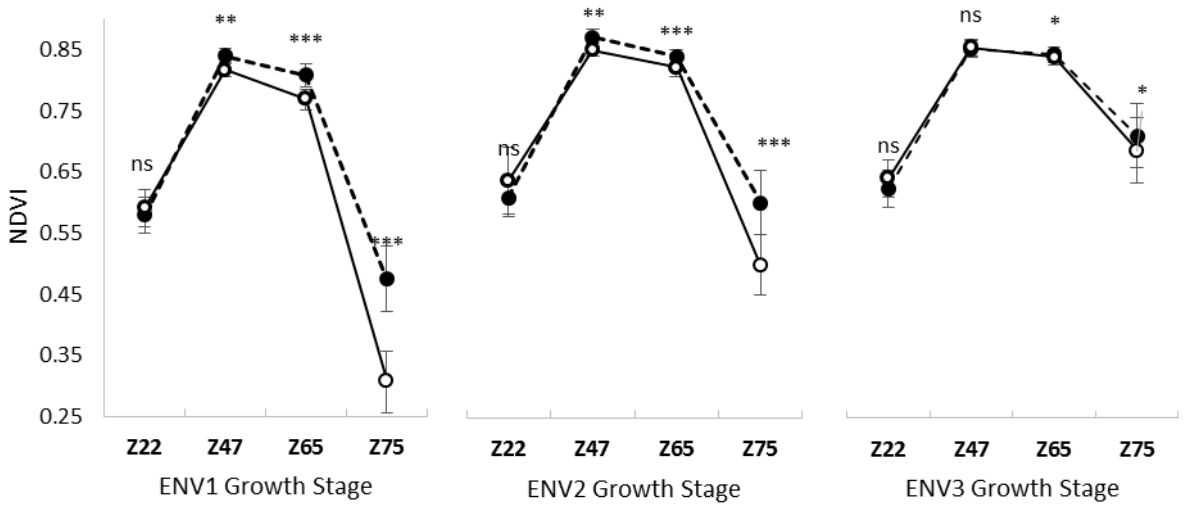


Figure 21. Variation in NDVI across growth stages Z22, Z47, Z65, and Z75 in parents Commander (●) and Compass (○) at each environment. Genotypic difference statistical significance is *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ns ($P > 0.05$).

There were no QTL detected for NDVI at tillering, but four QTL detected at Z47, five at Z65, and six at Z75 (Table 19). The QTL located on 6H was at a similar position in all three growth

stages and sowing dates, however it was stronger prior to flowering, explaining 6 - 9.9% of the variance at Z47 and 1.1 – 6.2% of the variance post flowering. A higher NDVI was associated with the Commander allele. The other QTL common across all growth stages Z47, Z65, Z75 was located on 2H (similar to QDAA_2H.1), but was more pronounced at Z75 explaining up to 29.1% of the variation compared to 9% at Z47. There was evidence of QTL x E interactions for this QTL with the Commander allele leading to higher values prior to anthesis at Z47 in ENV1, whereas the Compass allele was higher in ENV3. The effects of the other QTL can be found in Table 19. The QTL identified for kernel weight on 4H (QKW_4H) was in a similar region to NDVI prior to anthesis (QNDVIZ47) and not with NDVI either at or post anthesis or other developmental traits. QNDVIZ47_7H and QNDVIZ75_7H were also in a similar region to QKW_7H and QCano_7H, independent of developmental QTL.

Table 19. Summary of significant QTL detected for Canopy architecture (Cano), and NDVI at flag leaf sheath extension (NDVI47), Flowering (NDVIZ65), and Grain fill (Z75) for environments 1 - 3 (E1,E2,E3).

Trait	QTL Name	Sig Marker	Chr	Base pairs	_log10 (P)	QTLxE	Additive Effect			Variance (%)			Positive allele		
							E1	E2	E3	E1	E2	E3	E1	E2	E3
Canopy Architecture	QCano_2H	TP71853	2	27377053	45.9	no	0.3	0.3	0.3	20.4	20.4	23.2	Cdr	Cdr	Cdr
	QCano_3H	TP79676	3	665418658	4.1	no	0.2	0.2	0.2	7.9	7.9	9	Cmp	Cmp	Cmp
	QCano_6H.1	TP10195	6	16151120	18.0	yes	0.2	0.2	0.1	9.1	9.1	0.2	Cdr	Cdr	Cdr
	QCano_6H.2	TP50731	6	92522678	5.3	no	0.2	0.2	0.2	9.4	9.4	10.6	Cdr	Cdr	Cdr
	QCano_6H.3	TP75532	6	397073995	5.5	no	0.2	0.2	0.2	4.4	4.4	5	Cdr	Cdr	Cdr
	QCano_7H	TP6783	7	623251276	4.4	no	0.1	0.1	0.1	1.4	1.4	1.6	Cdr	Cdr	Cdr
NDVIZ47	QNDVIZ47_2H	TP72494	2	29447820	4.4	yes	0.002	_	0.004	2.1	_	9.2	Cdr	_	Cmp
	QNDVIZ47_4H	TP31945	4	4223709	3.4	no	0.001	0.001	0.001	1.4	1.8	1.1	Cdr	Cdr	Cdr
	QNDVIZ47_6H	TP58693	6	11573605	14.5	no	0.003	0.003	0.003	7.5	9.9	6	Cdr	Cdr	Cdr
	QNDVIZ47_7H	TP83955	7	604533307	4.2	no	0.002	0.002	0.002	1.9	2.5	1.5	Cdr	Cdr	Cdr
NDVIZ65	QNDVIZ65_2H.1	TP52698	2	28831852	3.5	yes	0.003	_	0.003	3.1	_	6.1	Cdr	_	Cmp
	QNDVIZ65_2H.2	TP49872	2	740677572	5.7	yes	0.004	_	_	5.2	_	_	Cmp	_	_
	QNDVIZ65_5H.1	TP80174	5	39723513	5.0	yes	0.004	_	_	5.2	_	_	Cmp	_	_
	QNDVIZ65_5H.2	TP10995	5	397048599	2.6	no	0.002	0.002	0.002	1.7	2.8	3.8	Cmp	Cmp	Cmp
	QNDVIZ65_6H	TP58693	6	11573605	14.0	no	0.004	0.004	0.004	5.4	9	12.3	Cdr	Cdr	Cdr
NDVIZZ75	QNDVIZ75_2H	TP52698	2	28831852	44.9	yes	0.04	0.019	_	29.1	14.5	_	Cdr	Cdr	_
	QNDVIZ75_3H	TP15416	3	13969384	4.2	no	0.007	0.007	0.007	0.9	2	5.1	Cdr	Cdr	Cdr
	QNDVIZ75_5H.1	TP20183	5	175762015	4.3	no	0.007	0.007	0.007	1	2.3	5.9	Cmp	Cmp	Cmp
	QNDVIZ75_5H.2	TP85294	5	567042991	2.3	no	0.005	0.005	0.005	0.5	1.2	3.1	Cdr	Cdr	Cdr
	QNDVIZ75_6H	TP87103	6	10693216	4.0	no	0.008	0.008	0.008	1.1	2.4	6.2	Cdr	Cdr	Cdr
	QNDVIZ75_7H	TP55882	7	62023891	2.9	no	0.006	0.006	0.006	0.6	1.4	3.5	Cdr	Cdr	Cdr

Developmental effect on yield, kernel weight, and canopy QTL

QTL for kernel weight (QKW_2H), grain yield (QYld_2H.1), grain number (QGN_2H), canopy architecture (QCano_2H, QNDVI_2H), Zadoks (QZad_2H), and days to awn appearance (QDAA_2H.1) were collocated in a similar region on chromosome 2H in ENV1 and ENV2 near the major photoperiod response developmental gene *HvPpd_H1* located at 29123724 base pairs. This is considered the candidate gene for the many QTL located near here. The PCA plots highlight the strong influence of QDAA_2H.1 on days to heading and the pleiotropic association with grain yield, canopy architecture, NDVI and kernel weight in sowing times ENV1 and ENV2 and the lack of relationship in ENV3 (Figure 22). In ENV1 and ENV2 a longer duration to awn appearance is positively associated with canopy traits and grain yield from the Commander QDAA_2H allele. Kernel weight was inversely related to NDVI and Canopy measurements prior to and post flowering in all environments , and particularly in ENV3 (Figure 22c) the scores and loadings suggests it is possible to achieve a relatively high kernel weight within both Compass and Commander QDAA_2H alleles, due to differences in canopy and kernel weight QTL.

Epistatic interactions

Interactions between pair of QTLs were only found for days to awn emergence and kernel weight. These interactions are presented in detail in Supplementary Table 11. Two of the four interactions involved the QTL linked to QDAA_2H, QZAD5H.1, and QKW_. The interaction with environment was not significant.

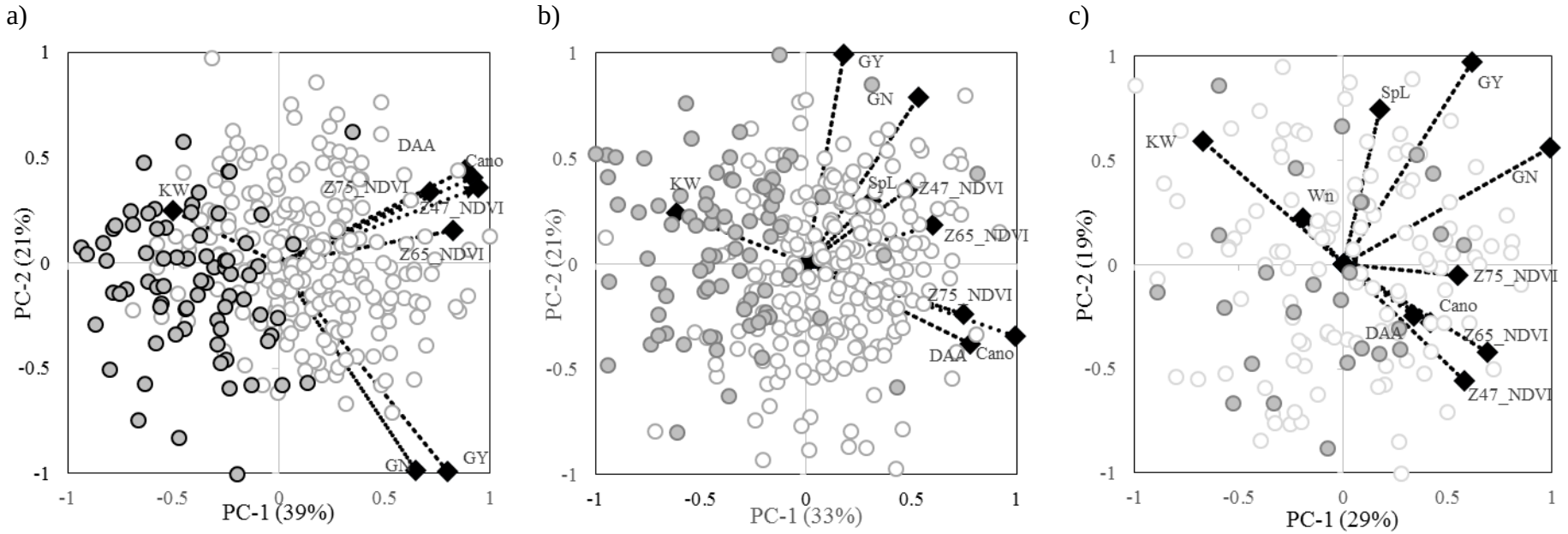


Figure 22. PCA plot of a) ENV1, b) ENV2, c) ENV3 for grain yield (GY), days to awn appearance (DAA), kernel weight (KW), grain number (GN), NDVI, Spike length (SpL), Waddington (Wn) open circles are Commander QDAA_2H alleles and shaded circles are QDAA_2H Compass alleles.

Discussion:

The objective of this study was to identify the genetic basis of improved yield and adaptation of Compass compared to Commander barley by developing a mapping population from inter - crossing these two well adapted and highly related genotypes. The major discussion for this paper will focus on the key loci controlling crop phenology in Compass barley due to the fact these genes play a major role in heading date and grain yield and other important traits.

Crop Development

These results have demonstrated Compass has a developmental pattern different to Commander: Compass has a shorter mean duration to awn appearance and awn primordia at May sowing dates and little difference in development from June sowing. Analysis of the Commander x Compass mapping population revealed a QTL for development and days to awn appearance on chromosome 2H in the May sowing dates of ENV1 and ENV2 near the major photoperiod response developmental gene HvPpd_H1 which is the likely candidate for this QTL (Turner et al., 2005). Our data shows the Commander allele at QDAA_2H results in greater photoperiod sensitivity than Compass. The importance of this locus for crop adaptation is not new, however, in the context of adaptation to Australian low - medium rainfall environments this is a new finding as it is a shift away from the photoperiod responsive Australian ideotypes as suggested by (Boyd et al., 2003). At the time of the review by Boyd et al., (2003) the minimal response to extended photoperiod among introductions from Europe and Canada was suggested to be of adaptive value in their respective regions and of limited value for Australia.

The recent study of Australian elite cultivars conducted by (Obsa et al., 2016) found parental lines Commander, Fleet, and WI4304 exhibited a relatively narrow range of phenology and also found the major developmental gene *Ppd_H1* was not associated with maturity variation in these populations. This is a contrast to our results where different alleles in the Compass x Commander population located near the *Ppd_H1* were the major regulator of development. Landraces from south-west Asia, southern Europe, and the Mediterranean basin have the *Ppd_H1* allele that confers early flowering under long days. Whereas the photoperiodic insensitive *ppd_H1* allele is present in landraces from central and northern Europe. This suggests the other parent of Compass, County, is likely to carry the insensitive *Ppd_H1* allele, in these environments the reduced response to photoperiod of *Ppd_H1* allows spring sown

plants to extend the period of vegetative growth and accumulate additional biomass that supports higher yields (Turner et al., 2005).

The studies conducted by Obsa et al. (2016) were sown at planting dates after May the 20th which resemble phenological environments similar to ENV3 (Jun sowing) in our study where the QZAD_2H effect on development was also not significant. Nonetheless, based on heading dates under short and long days, almost all commercial well adapted spring releases in Australia resemble the Commander type and possess a relatively short duration to heading in summer nurseries and very strong response to increase in photoperiod that facilitates sowing in May to June (Boyd et al., 2003). However, Compass demonstrates it is possible to achieve this development pattern without relying on a strong photoperiod response.

Photoperiod is generally considered to accelerate flowering in response to long days of >12 h of light per day (Turner et al., 2005). Of note is the fact that the effects of developmental QTL and QDAA_2H QTL in this study are occurring under minimal shifts in mean photoperiods. There was only a small increase in photoperiod of 0.1, 0.2 hours respectively from ENV1, and ENV2 well below 12 hr days during the early development period. This suggests that either QDAA_2H is very responsive to very small changes in photoperiod or there is an interaction with other environmental cues or QTL during the period from sowing to awn primordia. Digel et al. (2015) did suggest *Ppd_H1* does not have a strong effect on the vegetative to reproductive phase change. It is worth noting the decline in mean minimum temperatures from ENV1 to ENV3 during this period. Temperature effects could be equal to or greater than responses recorded for vernalisation or photoperiod (Read et al., 2003) when grown under milder winter growing conditions. This begins to build on the environmental modelling where we proposed lower mean minimum temperatures occurring under short photoperiods might be just as important as the effect of increasing daylength for the control of development of both Compass and Commander in the vegetative phase (Chapter 3).

In addition, other development QTL were identified. For the QTL on 6H (QDAA_6H.2), the Compass allele contributed to faster development under short photoperiods in ENV1 and ENV2 (Figure 15). The difference in values at the same daylength in Figure 15 represents the effects of the other developmental control such as QDAA_6H.2. While our data are suggesting this locus is also affecting the photoperiod pathway, temperature response interactions cannot be ruled out. The 6H QTL is located near to Circadian clock response candidate genes that are known to affect the photoperiod pathway such as cryptochromes (cry1 and cry2) (Imaizumi and Kay, 2006) and HvPRR1/HvTOC1 (Campoli et al., 2012). Plant Circadian clocks have

an ability to perceive and integrate temperature cues, as well as play a role in photoperiod dependent flowering, therefore it is also a likely strong candidate for interactions between temperature and photoperiod (Ford et al., 2016). Developmental QTL identified on 3H (QZad_3H) and 5H (QZad_5H.2) were consistent in all environments including the summer nursery, suggesting independence from photoperiod and temperature responses. However, the QZad_5H.1 interacted with QZad_2H under short photoperiods. The importance of these loci should not be understated as it means breeders can fine tune development under very small changes in photoperiod and have been successful in doing so with the release of Compass.

Results of this study challenge the current breeding methodology for adaptation to Australian environments. Breeders and physiologist have long favoured selection for a short BVP, to ensure the reproductive phase, and grain fill do not occur during sub optimal conditions (Boyd et al., 2003) when planted in Autumn. BVP is measured under saturated photoperiod and is often based on surrogate measures of floral initiation such as the timing to awn peep. To select a cultivar with a short BVP, breeders have made early generation selections over long days in summer nurseries for genotypes that flower early effectively selecting for increased photoperiod sensitivity. However, the success of Compass challenges this theory as it develops slower than Commander over summer but is quicker to both awn primordia and awn appearance in all commercially relevant autumn and winter sowing dates (Figure 16). The BVP measurement assumes that the timing of anthesis strongly reflects the timing of floral initiation and that photoperiod responses are not independent of one another throughout any of the other sub phases of development. This may not always be the case as there is lack of a clear correlation between early development and heading date in some circumstances (Vanoosterom and Acevedo, 1992). Furthermore, within the subset data the genotypes with the Compass developmental (QDAA_2H QTL) allele reached awn primordia quicker than the Commander allele in both sowing environments ENV1 and ENV2. This supports the literature that the *PpD_H1* locus is also a key regulator of inflorescence development (Turner et al., 2005). There is a correlation between early development and awn appearance in this study mostly due to the QDAA_2H.1 QTL (Figure 17) however; there is evidence of independent segregation associated with minor developmental QTL. The QTL detected for early inflorescence development in ENV3 (QWn_2H) was identified on the long arm of 2H and importantly was not previously detected for DAA or any other trait. This is located near to the *APETALA2* (*HvAP2*) gene that is known to control inflorescence development (Houston et

al., 2013). It is therefore possible to combine alternate development alleles to achieve diversity in early development for further exploitation

Linking crop development to yield

Across the National Variety Trials series and our previous data (chapter 2) it has been demonstrated that Compass consistently yields more than Commander, due to a greater kernel weight and a modest improvement in grain number resulting from increased grains per spike irrespective of flowering time. This is despite Compass flowering similar to or earlier than Commander over the normal range of sowing times. It was expected to find significant yield QTL independent of flowering time QTL. However, these were limited in the population studied, suggesting either that yield is not independent of phenology or that the environments experienced in this trial were not suited to Compass expressing its yield potential. QTLs for grain yield independent of crop development in barley still remain a challenging target due to large QTL × E interactions (Romagosa et al., 1999). We identified five QTL for grain yield in this study however all five had low LOD scores, low heritability and were subject to a QTL x E interaction.

While in this mapping population there was no significant phenotypic relationship between DAA and grain yield in ENV2 and ENV3, the PCA plots highlight the strong influence of the major photoperiod response QDAA_2H and the pleiotropic association with grain yield, canopy architecture, NDVI, and kernel weight. From the early May sowing in ENV1 a longer time to awn appearance had a small but significance positive association with grain yield. This was an unexpected result, and may be partly explained by the above average rainfall received in spring favouring later maturing genotypes, conditions that are not typical in southern Australia. Commercial plantings of barley in southern Australia are now moving towards early to midday planting dates. To our knowledge, this study is one of the first to identify developmental traits associated with yield from early May plantings, it has previously been suggested different development patterns may be required for early sowing (Young and Elliott, 1994). While Compass is higher yielding than Commander in most environments of southern Australia, these data highlight additional complexities including that a delay in flowering time associated with increased photoperiod sensitivity, such as occurs in Commander, may be beneficial for earlier sowing in favourable high yielding environments. This needs further investigation from earlier sowing times. Selection for developmental traits from this study may be more reliable than the short term yield performance data as the latter approach may

tend to favour genotypes that fail to capitalise on favourable growing conditions in some seasons, or are unable to satisfactorily complete their life-cycle in others (Boyd et al., 2003). Particularly as Compass has proven its performance in most of the variable climates of Australia, fulfilling the requirement for a short mean duration to flower to achieve a narrow flowering window from May sowing. Prior to 2016 and this study, Compass had not been tested in official trials in environments greater than 6 t/ha. It is therefore with some caution that the yield results are interpreted and more evaluation for this population in <6 t/ha yield environments is needed. It is for these reasons that much of the discussion in this paper is focused on the QTL related with the traits kernel weight and crop development, and while these were less correlated to yield than previous experience, they were highly heritable and relevant for southern environments.

Kernel weight and spike length

There were two major kernel weight QTL with high heritability (QKW_4H, and QKW_6H loci) that were independent of phenology traits, which demonstrated it was possible to improve kernel weight within cultivars of similar heading times. Figure 18 illustrates the positive additive effects contributed from the Compass alleles at these QTLs. On average, the Compass alleles at these QTL resulted in a mean increase in kernel weight of 6%, which can be achieved independent of the major development QTL located at 2H. It is therefore possible to select lines with improved kernel weight even where photoperiod sensitivity may be desired for early sowing.

The period of growth from awn primordia to tipping has been suggested as the most critical growth phase for determining grain number. Extending the length of the phase with differing phenology genes could be promising for improving yield as it is directly related to spikelet survival and grain yield per main spike (Alqudah and Schnurbusch, 2014). Neither the Compass nor Commander allele at QWn_2H that influenced inflorescence development was linked to spikelet length or number. The variant of PpD_H1 that decreased photoperiod sensitivity has previously been shown to increase the number of seeds per spike under favourable conditions (Digel *et al.*, 2015). While our study showed QDAA_2H photoperiod QTL had an effect on spike length, in ENV2 and ENV3 the effect of the 6H spike length QTL were greater and the addition of the Compass allele resulted in a 3 – 6mm increase in spike length, irrespective of the major Ppd 2H allele that equates to 1 - 2 more grains per spike (Figure 20). This may help explain the improved yield of Compass. This is an important finding as it

means breeders can manipulate the number of grains per spike within contrasting photoperiod sensitivity groups.

Canopy related traits

The flag leaf of Compass was more erect than that of Commander. This trait was under strong genetic control with a major QTL identified near the development QTL (QDAA.2H.1) on 2H but also on other linkage groups linked to almost all major development QTL. Pleiotropic effects of flowering time regulators on canopy related traits might be a consequence of changes in source - sink relationships triggered by the transition from vegetative to reproductive growth or inflorescence growth. Recent studies have indicated that leaf size is controlled by Ppd_H1 and photoperiod - dependent progression of plant development (Digel et al., 2016). The coordination of leaf growth with flowering may be part of a reproductive strategy to optimize resource allocation to the developing inflorescences and seeds (Digel et al., 2016) leading to improved kernel weight and or greater grains per spike. There is evidence in this study to suggest changes in the canopy, such as a more erect structure and those observed by NDVI, contributes to the improved kernel weight of Compass particularly as every kernel weight QTL was collocated with either the canopy architecture score or NDVI from leaf extension to grain fill. The more erect leaf may allow better light penetration into the canopy at a critical period of growth. The strong QTL identified for kernel weight on 4H was in a similar region to NDVI prior to anthesis (QNDVI47) and not with NDVI at or post anthesis. The effect of the QDAA_2H (QNDVI75_2H) allele had a larger influence on NDVI during grain fill than any other growth stage whereas the 6H QTL and the 7H QTL were more pronounced prior to anthesis. Maintenance of green colour (stay green) has been proposed as an important trait for improved grain plumpness by prolonging photosynthesis (Thomas et al., 2014), however despite ideal growing conditions in 2016 in this study there is limited evidence to suggest this improves kernel weight because all alleles associated with greater greenness as measured by NDVI were from Commander. Nonetheless the study demonstrated the strong link between senescence and development QTL that delay flowering time. Senescence is recognised as an adaptive strategy used by plants to respond to seasonal environmental cues such as changes in photoperiod (Thomas et al., 2014).

Early vigour has been proposed as a trait to ensure rapid development of leaf area, thereby reducing soil evaporative demand and improving yield (Tyagi et al., 2011). However, we found no significant variation or QTL for early vigour based on NDVI during tillering that would

suggest Compass has an advantage over Commander. Based on NDVI, changes in resource allocation prior to anthesis maybe an important trait for further integration, particularly as a more erect canopy structure was also linked to increased spike length on chromosome 6H. This may be associated with a better light environment prior to anthesis during the critical spike growth period and an erect canopy improving photosynthetic efficiency under high yielding conditions.

While we have focused mostly on traditional ideotype traits; spike length, kernel number and weight, and phenology; these observations highlight the possibility for relationships between canopy traits such as leaf erectness, spikes and stems that could modify the relative contribution of different yield components to final yield particularly during the pre - anthesis period. This requires more evaluation in the low - medium rainfall environments experienced in southern Australian drought prone environments and may in fact describe more of the yield improvement in Compass than phenology *per se*.

Conclusion:

The major difference between Compass and Commander controlling crop development is the response to photoperiod associated with the *HvPpd1* candidate gene on Chr2H. The reduction in the photoperiod response is a shift away from the traditional Australian ideotype and breeders can now consider selecting for a diverse range of phenology types in summer nurseries and introgress lines with Ppd insensitivity. A QTL on 6H also contributed to faster development under shorter days along with other developmental QTL independent of temperature and phenology. These QTLs provide scope to further fine tune development under very small changes in photoperiod. Two QTL for kernel weight on 4H and 6H were independent of phenology traits with high heritability and demonstrated it was possible to improve kernel weight within cultivars of similar heading date but different photoperiod response. Furthermore, major QTL were identified that contributed to canopy architecture, NDVI and kernel weight along with additional QTL that contributed to spike length could explain improvements in grain per spike and kernel weight of Compass as a result of improved resource allocation. To our knowledge, this is the first information on the genetic basis of phenology and yield related traits in elite cultivars conducted within the range of sowing dates over which barley is currently sown in the medium rainfall region of South Australia. The alleles discovered here are relevant as they control yield and adaptive traits of elite barley lines in the Mediterranean type environment of South Australia.

References:

Alqudah, A.M., Schnurbusch, T., 2014. Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. Functional Plant Biology 41, 424_436.

Alqudah, A.M., Sharma, R., Pasam, R.K., Graner, A., Kilian, B., Schnurbusch, T., 2014. Genetic Dissection of Photoperiod Response Based on GWAS of Pre_Anthesis Phase Duration in Spring Barley. PLoS ONE 9, e113120.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J Mol Biol 215, 403_410.

Araus, J.L., Slafer, G.A., Royo, C., Serret, M.D., 2008. Breeding for Yield Potential and Stress Adaptation in Cereals. Critical Reviews in Plant Sciences 27, 377_412.

Baum, M., Grando, S., Backes, G., Jahoor, A., Sabbagh, A., Ceccarelli, S., 2003. QTLs for agronomic traits in the Mediterranean environment identified in recombinant inbred lines of the cross 'Arta' × H. spontaneum 41_1. Theoretical and Applied Genetics 107, 1215_1225.

Bezant, J., Laurie, D., Pratchett, N., Chojecki, J., Kearsey, M., 1996. Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (Hordeum vulgare L.) cross. Heredity 77, 64.

Börner, A., Buck_Sorlin, G.H., Hayes, P.M., Malyshev, S., Korzun, V., 2002. Molecular mapping of major genes and quantitative trait loci determining flowering time in response to photoperiod in barley. Plant Breeding 121, 129_132.

Boyd, W.J.R., Li, C.D., Grime, C.R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Kamali, M.R.J., Barr, A.R., Moody, D.B., Lance, R.C.M., Logue, S.J., Raman, H., Readj, B.J., 2003. Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. Australian Journal of Agricultural Research 54, 1277_1301.

Campoli, C., Shtaya, M., Davis, S.J., von Korff, M., 2012. Expression conservation within the circadian clock of a monocot: natural variation at barley Ppd_H1 affects circadian expression of flowering time genes, but not clock orthologs. BMC Plant Biol 12, 97.

Cockram, J., Chiapparino, E., Taylor, S.A., Stamati, K., Donini, P., Laurie, D.A., O'Sullivan, D.M., 2007. Haplotype analysis of vernalization loci in European barley germplasm reveals novel VRN_H1 alleles and a predominant winter VRN_H1/VRN_H2 multi_locus haplotype. Theoretical and Applied Genetics 115, 993_1001.

Coventry, S.J., Barr, A.R., Eglinton, J.K., McDonald, G.K., 2003. The determinants and genome locations influencing grain weight and size in barley (<emph type="2">Hordeum vulgare</emph> L.). Australian Journal of Agricultural Research 54, 1103_1115.

Digel, B., Pankin, A., von Korff, M., 2015. Global Transcriptome Profiling of Developing Leaf and Shoot Apices Reveals Distinct Genetic and Environmental Control of Floral Transition and Inflorescence Development in Barley. Plant Cell.

Digel, B., Tavakol, E., Verderio, G., Tondelli, A., Xu, X., Cattivelli, L., Rossini, L., von Korff, M., 2016. Photoperiod_H1 (Ppd_H1) Controls Leaf Size. Plant Physiol 172, 405_415.

Faure, S., Turner, A.S., Gruszka, D., Christodoulou, V., Davis, S.J., Von Korff, M., Laurie, D.A., 2012. Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (Hordeum vulgare) to short growing seasons. Proceedings of the National Academy of Sciences of the United States of America 109, 8328_8333.

Ford, B., Deng, W., Clausen, J., Oliver, S., Boden, S., Hemming, M., Trevaskis, B., 2016. Barley (Hordeum vulgare) circadian clock genes can respond rapidly to temperature in an EARLY FLOWERING 3 _dependent manner. Journal of Experimental Botany 67, 5517_5528.

Forsythe, W.C., Rykiel, E.J., Stahl, R.S., Wu, H._i., Schoolfield, R.M., 1995. A model comparison for daylength as a function of latitude and day of year. Ecological Modelling 80, 87_95.

Fu, D., Szűcs, P., Yan, L., Helguera, M., Skinner, J.S., von Zitzewitz, J., Hayes, P.M., Dubcovsky, J., 2005. Large deletions within the first intron in VRN_1 are associated with spring growth habit in barley and wheat. Molecular Genetics and Genomics 273, 54_65.

Gallagher, L.W., Soliman, K.M., Vivar, H., 1991. Interactions among loci conferring photoperiod insensitivity for heading time in spring barley. Crop Science 31, 256_261.

Houston, K., McKim, S.M., Comadran, J., Bonar, N., Druka, I., Uzrek, N., Cirillo, E., Guzy_Wrobelska, J., Collins, N.C., Halpin, C., Hansson, M., Dockter, C., Druka, A., Waugh, R., 2013. Variation in the interaction between alleles of HvAPETALA2 and microRNA172 determines the density of grains on the barley inflorescence. Proceedings of the National Academy of Sciences 110, 16675_16680.

Imaizumi, T., Kay, S.A., 2006. Photoperiodic control of flowering: not only by coincidence. Trends in Plant Science 11, 550_558.

Karsai, I., Szűcs, P., Kőszegi, B., Hayes, P.M., Casas, A., Bedő, Z., Veisz, O., 2008. Effects of photo and thermo cycles on flowering time in barley: a genetical phenomics approach. Journal of Experimental Botany 59, 2707_2715.

Laurie, D.A., Pratchett, N., Bezant, J.H., Snape, J.W., 1994. Genetic_analysis of a photoperiod response gene on the short arm of chromosome 2(2h) of hordeum_vulgare (barley). Heredity 72, 619_627.

Laurie, D.A., Pratchett, N., Snape, J.W., Bezant, J.H., 1995. RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (Hordeum vulgare L.) cross. Genome 38, 575_585.

Li, J., Ji, L., 2005. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity (Edinb) 95, 221_227.

Lu, F., Lipka, A.E., Glaubitz, J., Elshire, R., Cherney, J.H., Casler, M.D., Buckler, E.S., Costich, D.E., 2013. Switchgrass Genomic Diversity, Ploidy, and Evolution: Novel Insights from a Network_Based SNP Discovery Protocol. PLoS Genetics 9, e1003215.

Major, D.J., 1980. Photoperiod response characteristics controlling flowering of nine crop species. Canadian Journal of Plant Science 60, 777_784.

Mansour, E., Casas, A.M., Gracia, M.P., Molina_Cano, J.L., Moralejo, M., Cattivelli, L., Thomas, W.T.B., Igartua, E., 2014. Quantitative trait loci for agronomic traits in an elite barley population for Mediterranean conditions. Molecular Breeding 33, 249_265.

Mascher, M., Gundlach, H., Himmelbach, A., Beier, S., Twardziok, S.O., Wicker, T., Radchuk, V., Dockter, C., Hedley, P.E., Russell, J., Bayer, M., Ramsay, L., Liu, H., Haberer, G., Zhang, X.Q., Zhang, Q., Barrero, R.A., Li, L., Taudien, S., Groth, M., Felder, M., Hastie, A., Simkova, H., Stankova, H., Vrana, J., Chan, S., Munoz_Amatriain, M., Ounit, R., Wanamaker, S., Bolser, D., Colmsee, C., Schmutzer, T., Aliyeva_Schnorr, L., Grasso, S., Tanskanen, J., Chailyan, A., Sampath, D., Heavens, D., Clissold, L., Cao, S., Chapman, B., Dai, F., Han, Y., Li, H., Li, X., Lin, C., McCooke, J.K., Tan, C., Wang, P., Wang, S., Yin, S., Zhou, G., Poland, J.A., Bellgard,

M.I., Borisjuk, L., Houben, A., Dolezel, J., Ayling, S., Lonardi, S., Kersey, P., Langridge, P., Muehlbauer, G.J., Clark, M.D., Caccamo, M., Schulman, A.H., Mayer, K.F.X., Platzer, M., Close, T.J., Scholz, U., Hansson, M., Zhang, G., Braumann, I., Spannagl, M., Li, C., Waugh, R., Stein, N., 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature 544, 427_433.

Obsa, B.T., Eglinton, J., Coventry, S., March, T., Langridge, P., Fleury, D., 2016. Genetic analysis of developmental and adaptive traits in three doubled haploid populations of barley (Hordeum vulgare L.). Theoretical and Applied Genetics 129, 1139_1151.

Poland, J.A., Brown, P.J., Sorrells, M.E., Jannink, J._L., 2012. Development of High_Density Genetic Maps for Barley and Wheat Using a Novel Two_Enzyme Genotyping_by_Sequencing Approach. PLOS ONE 7, e32253.

Read, B.J., Raman, H., McMichael, G., Chalmers, K.J., Ablett, G.A., Platz, G.J., Raman, R., Genger, R.K., Boyd, W.J.R., Li, C.D., Grime, C.R., Park, R.F., Wallwork, H., Prangnell, R., Lance, R.C.M., 2003. Mapping and QTL analysis of the barley population Sloop × Halcyon. Australian Journal of Agricultural Research 54, 1145_1153.

Ren, X.F., Li, C.D., Boyd, W.J.R., Westcott, S., Grime, C.R., Sun, D.F., Lance, R., 2010. QTLs and their interaction determining different heading dates of barley in Australia and China. Crop & Pasture Science 61, 145_152.

Richards, R.A., 1991. Crop improvement for temperate australia _ future opportunities. Field Crops Research 26, 141_169.

Roberts, E.H., Summerfield, R.J., Cooper, J.P., Ellis, R.H., 1988. Environmental_control of flowering in barley (hordeum_vulgare_l) .1. Photoperiod limits to long_day responses, photoperiod_insensitive phases and effects of low_temperature and short_day vernalization. Annals of Botany 62, 127_144.

Rollins, J.A., Drosse, B., Mulki, M.A., Grando, S., Baum, M., Singh, M., Ceccarelli, S., von Korff, M., 2013. Variation at the vernalisation genes Vrn_H1 and Vrn_H2 determines growth and yield stability in barley (Hordeum vulgare) grown under dryland conditions in Syria. Theoretical and Applied Genetics 126, 2803_2824.

Romagosa, I., Han, F., Ullrich, S.E., Hayes, P.M., Wesenberg, D.M., 1999. Verification of yield QTL through realized molecular marker_assisted selection responses in a barley cross. Molecular Breeding 5, 143_152.

Teulat, B., Merah, O., Souyris, I., This, D., 2001. QTLs for agronomic traits from a Mediterranean barley progeny grown in several environments. Theoretical and Applied Genetics 103, 774_787.

Thomas, H., Ougham, H., Sadras, V., Calderini, D., 2014. Senescence and crop performance. Crop Physiology: Applications for Genetic Improvement and Agronomy, 223.

Tondelli, A., Francia, E., Visioni, A., Comadran, J., Mastrangelo, A.M., Akar, T., Al_Yassin, A., Ceccarelli, S., Grando, S., Benbelkacem, A., van Eeuwijk, F.A., Thomas, W.T.B., Stanca, A.M., Romagosa, I., Pecchioni, N., 2014. QTLs for barley yield adaptation to Mediterranean environments in the 'Nure' x 'Tremois' biparental population. Euphytica 197, 73_86.

Turner, A., Beales, J., Faure, S., Dunford, R.P., Laurie, D.A., 2005. Botany: The pseudo_response regulator Ppd_H1 provides adaptation to photoperiod in barley. Science 310, 1031_1034.

Tyagi, K., Park, M.R., Lee, H.J., Lee, C.A., Rehman, S., Steffenson, B., Lee, K.J., Yun, S.J., 2011. Diversity for seedling vigor in wild barley (Hordeum vulgare L. subsp. spontaneum) germplasm. Pakistan Journal of Botany 43, 2167_2173.

Vanoosterom, E.J., Acevedo, E., 1992. Adaptation of barley (hordeum_vulgare l) to harsh mediterranean environments .2. Apical development, leaf, and tiller appearance. Euphytica 62, 15_27.

Waddington, S.R., Cartwright, P.M., Wall, P.C., 1983. A quantitative scale of spike initial and pistil development in barley and wheat. Annals of Botany 51, 119_130.

Young, K.J., Elliott, G.A., 1994. Art evaluation of barley accessions for adaptation to the cereal growing regions of western_australia, based on time to ear emergence. Australian Journal of Agricultural Research 45, 75_92.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Research 14, 415_421.

Supplementary Tables and Figures

Supplementary Table 5 Partial replication design structure

	ENV1	ENV2	ENV3
No of genotypes	370	370	173
Connectivity within ENV (REPS)	146	146	86
Genotypic Connectivity with ENV1		158	103
Genotypic Connectivity with ENV2	158		103

Supplementary Table 6 Summary statistics based on BLUEs for W score, spike length (mm) and Canopy Structure for the population and variation in the parents across all sowing environments

	Waddington Score			Spike length			Canopy Structure		
	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3
Commander	3.9	3.5	3.6	71.6	68.9	73.4	2.8	2.4	2.2
Compass	4.3	4.3	4.3	63.9	77.1	78.6	1.0	1.0	1.1
Mean	4.1	3.8	3.9	73.1	78.9	78.7	1.7	1.7	1.6
Min	3.2	2.6	3.1	58.6	57.7	54.9	0.3	0.3	0.4
Max	5.1	4.7	4.8	86.7	100.7	93.0	4.3	4.4	3.7
No. obs	52	52	173	53	367	172	369	369	173
s.d.	0.5	0.4	0.2	6.0	5.9	5.9	0.7	0.7	0.7
F Pr	<0.001	<0.001	<0.001	0.01	<0.001	0.02	0.001	0.001	0.001

Supplementary Table 7: Summary of significant marker effects and environment effects for Waddington development score Scale (recorded when Compass was at W4) and spike length on the main culm the environment (only markers with probability level P<0.05 are shown, ns = P>0.05), Single marker effects are expressed as the mean of Compass allele subtracted from the mean of the Commander allele

				Effect Compass _ Commander		
	Marker (M1)	M1	M1 x Env	ENV1	ENV2	ENV3
Waddington (W)	QWn_2H	0.001	ns	_0.2	_0.1	_0.1
	QZad_2H	<0.001	<0.001	0.7	0.4	0.1
	QZad_3H	0.01	ns	0.1	0.2	0.1
	QZad_5H.1	<0.001	ns	_0.3	_0.1	_0.1
Spike length (mm)	QZad_2H	<0.001	0.01	_6.3	_2.5	_0.6
	QZad_6H	<0.001	ns	3.4	2.4	4.9
	QSPL_6H.1	<0.001	0.01	4.0	2.5	6.2
	QSPL_6H.2	<0.001	ns	3.5	3.1	5.7

Supplementary Table 8 Summary statistics for Zadoks growth stage for the population and variation in the parents across all sowing environments

	Summer Nursery	ENV1	ENV2	ENV3
Total number of values	597	369	368	173
Mean	61.3	63.4	63.6	65.7
Min	29.0	56.5	54.0	62.4
Max	73.0	74.4	73.6	70.4
Commander	65.8	61.7	61.2	65.0
Compass	46.8	69.0	68.1	66.4
Std Dev	9.1	2.8	3.7	1.3

Supplementary Table 9 Summary of significant QTL detected for growing degree days to awn appearance (GDDAA)

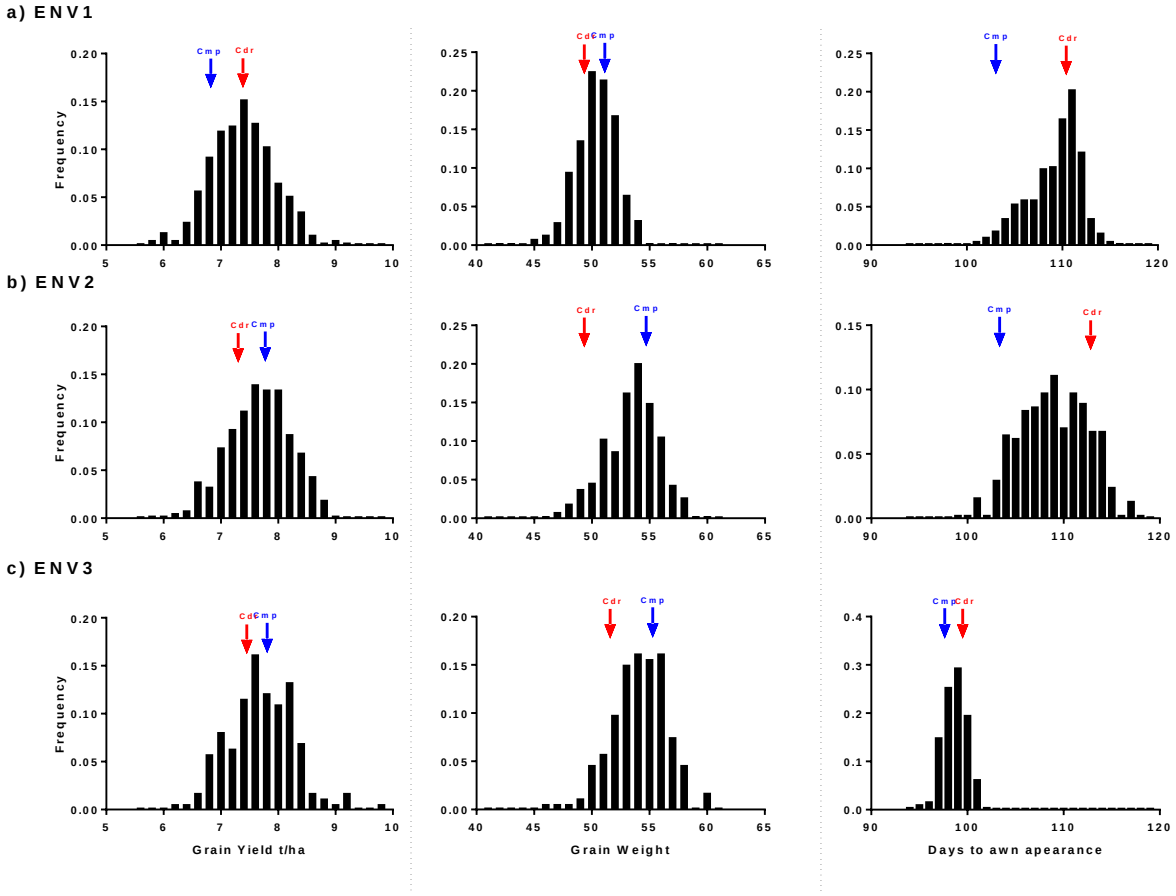
Trait	QTL Name	Sig Marker	Chr	Base pairs	_log10 (P)	QTL x E	Additive Effect			Variance (%)			Positive allele		
							E1	E2	E3	E1	E2	E3	E1	E2	E3
GDD AA	QGDDAA_2H	TP57409	2	29202919	144.0	yes	26.43	26.075	2.563	71.9	37.6	3.3	Cdr	Cdr	Cdr
	QGDDAA_3H	TP68560	3	4889	4.104	no	2.806	2.806	2.806	0.8	0.4	3.9	Cdr	Cdr	Cdr
	QGDDAA_5H.1	TP40204	5	219815755	4.11	no	2.91	2.91	2.91	0.9	0.5	4.2	Cmp	Cmp	Cmp
	QGDDAA_5H.2	TP85294	5	567042991	2.23	no	2.529	2.529	2.529	0.7	0.4	3.2	Cdr	Cdr	Cdr
	QGDDAA_6H.1	TP10034	6	9852710	2.797	no	2.469	2.469	2.469	0.6	0.3	3	Cdr	Cdr	Cdr
	QGDDAA_6H.3	TP75532	6	397073995	3.344	no	3.423	3.423	3.423	1.2	0.6	5.8	Cdr	Cdr	Cdr

Supplementary Table 10 Summary statistics based on BLUEs for NDVI at Z22, Z47, Z65, and Z75 for the population and variation in the parents across all sowing environments.

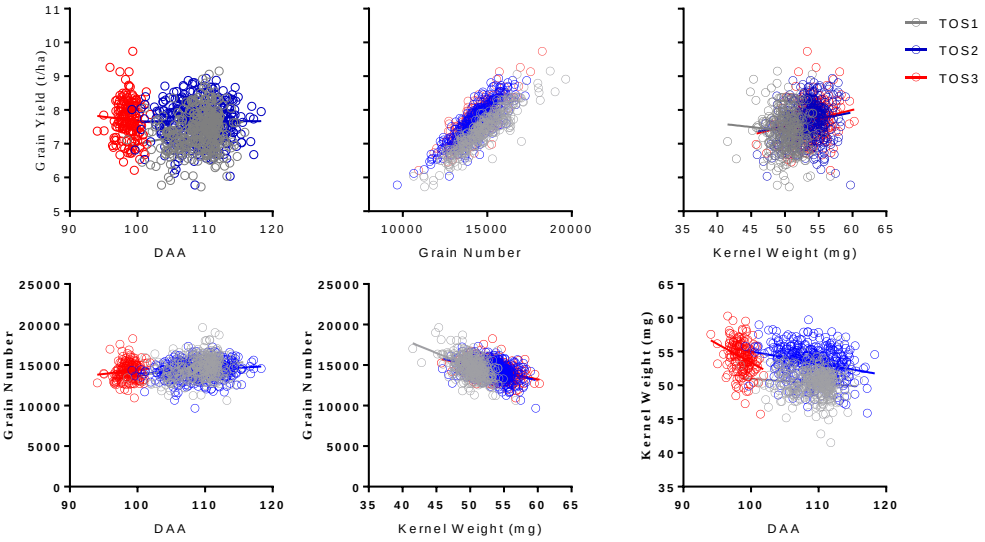
	NDVI GS22			Flag leaf extending			Flowering			NDVI GS75		
	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3	ENV1	ENV2	ENV3
Commander	0.58	0.61	0.62	0.84	0.87	0.85	0.81	0.84	0.84	0.48	0.60	0.7
Compass	0.59	0.64	0.64	0.82	0.85	0.85	0.77	0.82	0.84	0.31	0.50	0.7
Mean	0.58	0.58	0.64	0.83	0.86	0.85	0.80	0.83	0.84	0.43	0.58	0.7
Min	0.46	0.39	0.54	0.79	0.81	0.80	0.73	0.78	0.80	0.21	0.41	0.5
Max	0.68	0.73	0.73	0.87	0.88	0.88	0.85	0.87	0.88	0.64	0.71	0.8
s.d.	0.03	0.05	0.03	0.01	0.01	0.01	0.02	0.01	0.01	0.07	0.05	0.1
F Pr	<0.001	0.01	ns.	<0.001	<0.001	ns.	<0.001	<0.001	<0.001	<0.001	<0.001	0.02
Heritability	0.37	0.20	0.11	0.36	0.44	0.07	0.32	0.28	0.24	0.44	0.32	0.18

Supplementary Table 11, Summary of QTL interactions for kernel weight and days to awn appearance. Alleles Cdr = Commander, Cmp = Compass. Values followed by the same letter are not significantly different (LSD, P≤0.05)

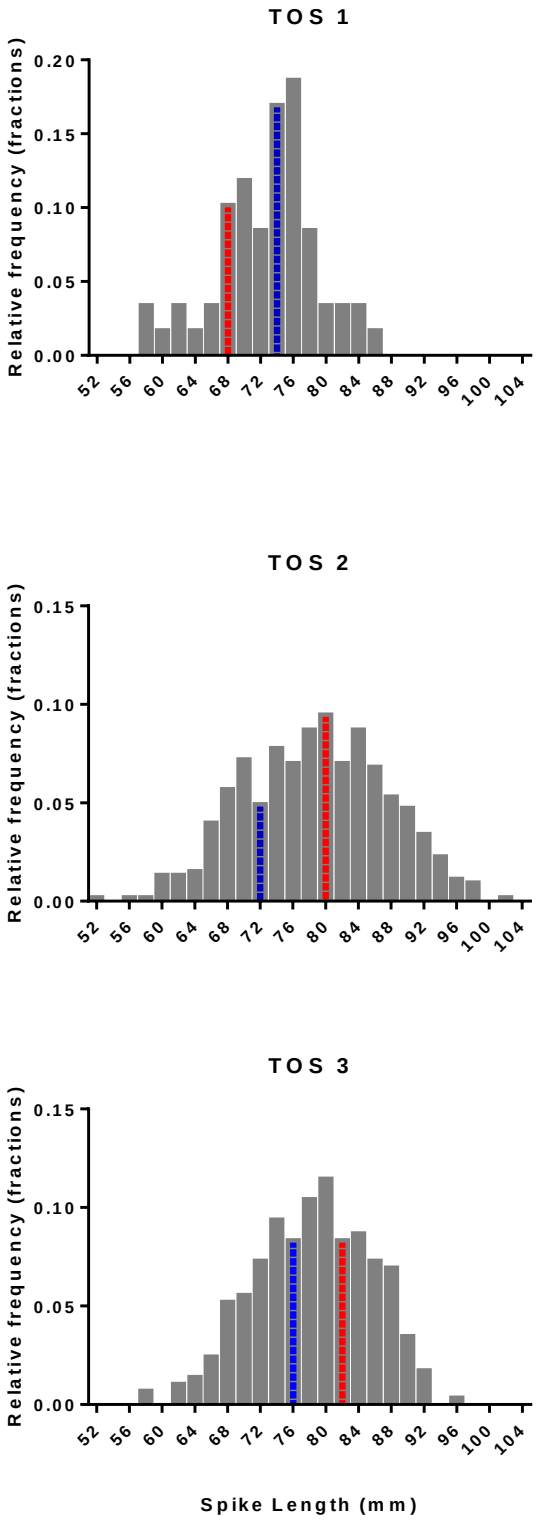
Loci, alleles		**Average**	
QKW_3H	QKW_7H	Kernel Weight (mg)	
1	1	51.6	a
1	2	52.08	a
2	1	51.66	a
2	2	53.21	b
QKW_2H	QKW_3H	Kernel Weight (mg)	
1	1	51.9	a
1	2	52.3	a
2	1	52.07	a
2	2	53.53	b
QZAD2H	QZAD5H.1	Days to Awn Appearance	
1	1	107.7	b
1	2	108.8	c
2	1	104	a
2	2	104.2	a
QZad_5H.1	QZad_5H.2	Days to Awn Appearance	
1	1	107.5	b
1	2	105.7	a
2	1	108.4	c
2	2	107.8	bc



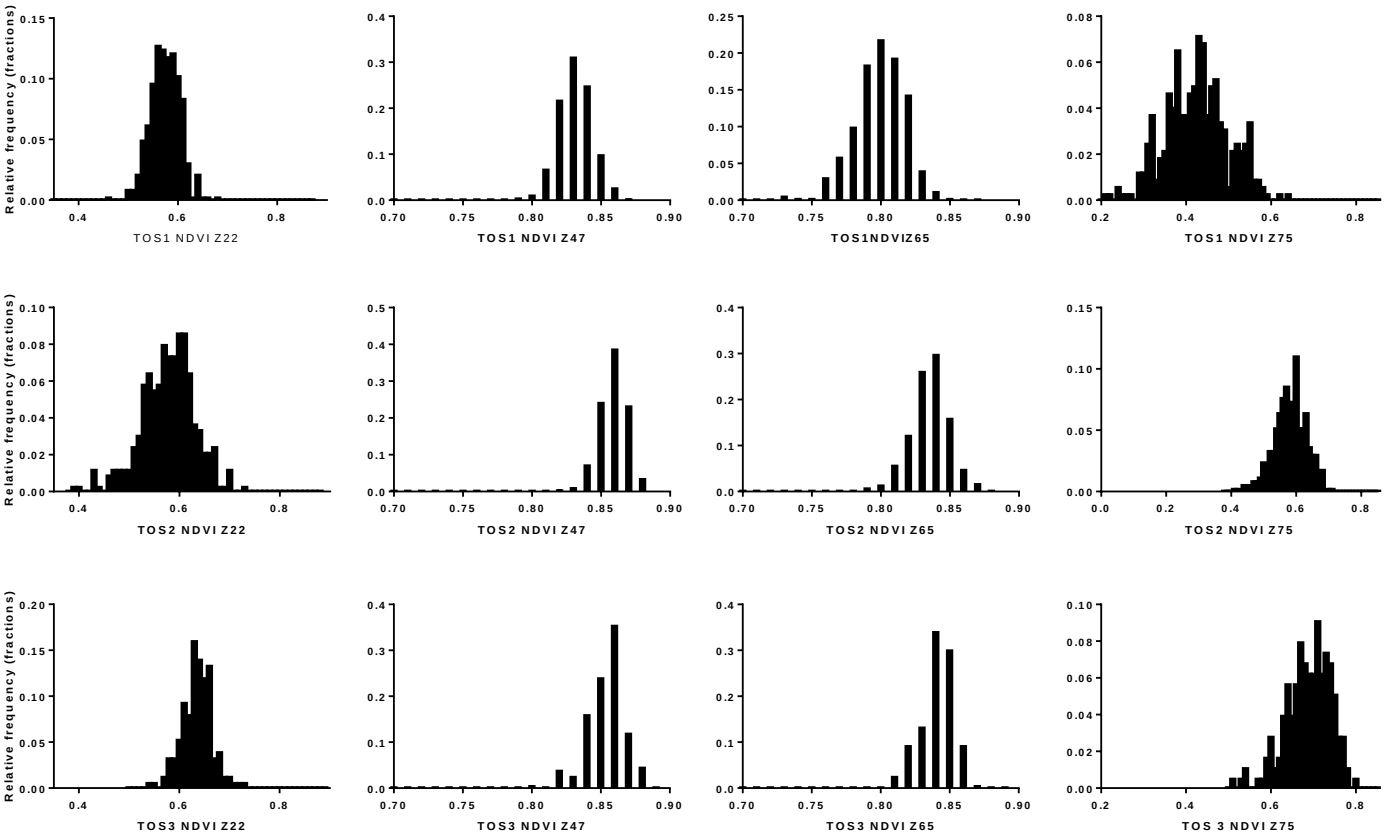
Supplementary Figure 10. Population frequency distribution for key agronomic traits grain yield, kernel weight, and days to heading for a) ENV1, b) ENV2, and c) ENV3. Mean trait values of the parents Commander (Cdr) and Compass (Cmp) are indicated by the arrows.



Supplementary Figure 11. Relationship agronomic traits days to awn appearance, grain number, kernel weight and grain yield in ENV1, 2, 3.



Supplementary Figure 12. Population frequency distribution for main culm spike length (mm) in a) ENV1, b) ENV2, and c) ENV3. The arrows indicate mean trait values of the parents Commander (CDR blue) and Compass (Cmp red).



Supplementary Figure 13. Population frequency distribution for NDVI at all three sowing times (TOS1 – 3) and growth stages (Z22, Z47, Z65, Z75)

CHAPTER 6: General Discussion, main findings and conclusions

General Discussion

The objective of this study was to identify the physiological and genetic basis of the 10% improved yield and adaptation of a newly released; barley variety, Compass, compared to the current malting benchmark variety Commander. Given the importance of crop development for yield and adaptation, the study focused on the key differences in crop development influencing yield in Commander and Compass. The significant yield improvement of Compass has been achieved despite Compass being genetically similar to and derived from Commander. Compass represents an important step change in yield and adaptation and the chapters in this thesis used phenotypic, environmental, and genotypic data to explain crop development and yield improvement in this elite barley line adapted to Australian conditions. This series of experiments demonstrated the power of using a multidisciplinary approach to understanding GxE and sowing time interactions. This thesis is a significant contribution to knowledge for the Australian barley industry, by explaining the development of cultivars adapted to Australian conditions and identifying opportunities to further increase yield potential. Time to heading has not been studied in this detail in Australian barley varieties for more than 15 years (Boyd et al., 2003) and the last detailed studies on pre anthesis traits were conducted by Kernich *et al.* (1997) in which Schooner was the latest commercially available variety. Schooner is now outclassed with yields at least 20% below Compass in most National Variety Trials (NVT). In 2017, Compass remains among the top three performing cultivars in South Australia (NVT Online). This general discussion reiterates many of the key points in the previous three chapters, identifies possible uncertainties and attempts to synthesise the main findings from the body of work concluding with recommendations for breeders and crop physiologists.

The first series of experiments described in Chapter 3 were designed to describe the major developmental differences between Compass and Commander at commercially relevant planting dates for southern Australia. This revealed a new finding for Australian environments; under field conditions, Compass was less responsive to photoperiod and had a shorter time to anthesis than Commander and Fathom when sown in late April and early-mid May. However, the three varieties did not differ from later planting dates, in June and July. The faster development of Compass compared to Commander from April and May sowing dates was unexpected, because previous breeding trials suggested Compass and Commander flowered at a similar time. However, most of the breeding trials prior to 2010, when Compass was being developed, were sown in late May – June. At these sowing dates, many of the major developmental differences between cultivars are not expressed; this is noted in Chapters 3, 4, and 5 from the later sowing dates. This finding in itself highlights the need to conduct experiments in representative environments. The fact many of the published literature is based on sowing dates from late May – July may be part of the reason that differences in

photoperiod responses have been masked previously. Photoperiod sensitivity has been widely regarded as a requirement for cultivars adapted to Australian conditions (Boyd *et al.*, 2003). However, our results show it is possible to achieve a short duration to flowering without relying as strongly on photoperiod response, therefore some of the previous assumptions regarding the major developmental control of cultivars adapted to Australian environments may be misleading. There was still variation in development between sites that could not be explained by photoperiod responsiveness and thermal time alone, which suggests other genetic and environmental factors that previously haven't been considered may be equally or more important in regulating anthesis time.

Given the strong G x time of sowing interaction for flowering time observed in Commander and Compass the studies in Chapters 3, 4 and 5 focused on understanding the major environmental and genetic controls of flowering. The key to improving crop adaptation will be to understand the cumulative effects of the environmental factors from sowing that trigger the complex biological processors that control flowering time. Application of partial least regression (PLS) was developed to explain flowering time and provides an important step towards improved biological understanding. The outputs from this model identified key phenological environments that could be targeted for genetic analysis of crop development in the Commander x Compass bi-parental mapping population in Chapter 5. This ensures maximum variation in development is exploited and the environmental drivers of development are adequately described.

The modelling approach developed in Chapter 3 has provided a methodology to identify the most relevant environmental variables that regulate crop development and helped to define phenology environments in southern Australia. Outcomes from this research provide new insight into G x sowing date interactions, and the temperature and photoperiodic responses that contribute to the adaptation of high yielding barley lines across commercially relevant sowing times. It was possible to fit both a complex and simplified model using PLS that described more than 90% of the variation in thermal time to anthesis. Data from the elite genotypes Compass, Commander and Fathom across 35 environments was fitted with an accuracy of between 22 and 47 °C.d (which equates to 3 – 4 days), similar to the model used in (Alzueta *et al.*, 2014). This confirms PLS and the methodology used in this study has application for robust characterisation of phenology in these environments, and is in agreement with other studies utilising PLS for phenology, for example chilling periods in walnuts (Luedeling and Gassner, 2012) and phenology in apricots (Guo *et al.*, 2015). While photoperiod had a large effect on development during the period just prior to flowering, this approach has helped quantify the effect of subtle changes in temperature on barley. It provided new evidence that the effect of minimum temperatures in the early phases of development may be of equal or greater importance than photoperiod in determining the total thermal time to anthesis. This has implications for breeders

striving to fine tune crop development under the shorter photoperiods and milder winter conditions experienced in South Australia.

The development of the Commander x Compass bi-parental mapping population allowed genetic analysis of the loci controlling developmental and yield traits, and was in some respects a final validation exercise. This population was developed within the PhD project and was completed over two years by single seed descent; however, this meant there was only enough seed for field trials in the final year of the PhD. In addition, due to budget constraints only a single site could be sown at Roseworthy. However the modelling undertaken in Chapter 3 directed the use of three different planting dates to create a temperature and photoperiod gradient. The major difference between Compass and Commander controlling crop development identified in genetic analysis in Chapter 5 was response to photoperiod associated with the HvPpd1 candidate gene on Chromosome 2H . This validated the GxE analysis in Chapter 3. This is a key finding as the reduced photoperiod response is a shift away from the traditional Australian developmental ideotype, which considered photoperiod sensitivity to be the major control of flowering time. Consequently, breeders can now consider selecting for a diverse range of phenology types in summer nurseries and introgress lines with Ppd insensitivity similar to Compass and European material such as County. It is understandable why there has been limited selection within breeding programs for this type of genotype, given the use of summer nurseries, glasshouse or growth room environments to speed up and select early generations, all leading to selection under long day conditions. However, as demonstrated by these studies this also has implications for phenology and suitable phenology types can be achieved with alternate selection approaches. While it the major difference between Compass and Commander is their response to photoperiod. The modelling in Chapter 3 described variation in flowering due to factors other than photoperiod, importantly there is QTL in Chapter 5 that were also consistent across all photoperiod environments. These begin to describe differences some of the genetic drivers for the differences in flowering time that was independent of photoperiod. In particular, a QTL on 6H also contributed to faster development under shorter days along. Other developmental QTL also influenced flowering time, independent of sowing date. These QTL provide scope to further fine tune development under very small changes in photoperiod, and their environmental control requires further investigation.

The effect of other environmental effects are an important finding given current crop simulation models such as APSIM do not account for many of these effects which are particularly important at earlier sowing dates. This highlights that many of the parameters for phenology in Australian barley cultivars may need re-calibrating and our study should be used to help inform improvements to current flowering time models. The genetic analysis in Chapter 5 has been successful in validating n

quantifying the effect of the Compass photoperiod allele needed to readjust models, and other development QTL effects. The PLS approach in Chapter 3 has proven its usefulness for characterising the effects of environmental variation. Combining these two approaches paves the way for development of a four-dimensional profile of crop science involving genotype (*G*), phenotype (*P*), envirotype (*E*) and time (*T*) (developmental stage) as proposed by (Xu, 2016).

The environmental and genetic control of anthesis of Compass and Commander were described in Chapter 3 and Chapter 5, but the link between the developmental pattern and yield was still not analysed. To address this issue, Chapter 4 dissected the variation in pre-anthesis phases of crop development using elite germplasm at a range of sowing times commonly used by farmers, while the second component of Chapter 5 associated phenology QTL with grain yield related traits and QTL. The findings of these analyses shed light on the variation in phenology and the pre-anthesis phases in barley cultivars adapted to southern Australia. Chapter 4 concluded a focus on anthesis date and grain yield was more important than pre-anthesis phenological traits and their plasticity. Grain yield development in barley is dynamic and yield components show considerable compensation among adapted cultivars. Consequently, high yielding adapted varieties achieve yield through multiple pathways. Focussing on one growth phase and its associated yield components may not necessarily result in improved yields because of compensatory changes in other yield components. Compared to Commander, the improved yield of Compass was derived from a combination of relatively small improvements in many traits, mainly by shortening all phenological phases. The greater yield of Compass was associated with slight increases in dry matter, harvest index, grain number per m^2, and consistently improved grain weight.

The genetic analysis in Chapter 5 revealed two QTL associated with kernel weight on 4H and 6H were independent of phenology traits with high heritability and demonstrated it was possible to improve kernel weight within cultivars of similar heading date but different photoperiod response and grain number. This suggests grain weight can be improved independently of grain number, and may provide scope for further yield improvement. This is a significant result as the QTL were stable across environments and partly explains the significant yield improvement observed in Compass.

The lack of significant and consistent QTL for grain yield was a little disappointing; however, this could be due to the environmental conditions experience in 2016 when the population was evaluated. The studies in 2014 and 2015 (Chapter 4) which described the major physiological differences between Compass and Commander were conducted in seasonal conditions typical of Mediterranean-like conditions experienced across southern Australian. However, the 2016 growing conditions were not consistent with long-term environmental data. This is further highlighted by the relative performance of Compass compared to Commander in SA NVT trials, in 2014 and 2015 when

Compass yielded more than 10% above Commander; however, in 2016 there was no significant difference. In the 2016 study (Chapter 5) genotypes that had a longer development cycle yielded higher than genotypes that were quicker meaning Commander was slightly higher yielding than Compass. This was an unexpected result and not consistent with findings from Chapter 4. This may be partly explained by the above average rainfall received in spring favouring later maturing genotypes, conditions that are not typical in southern Australia. Prior to 2016 and this study, Compass had not been tested in official trials in environments greater than 6 tonne/ha. It is therefore with some caution that the yield results are interpreted. It is for these reasons that much of the discussion in Chapter 5 is focused on the QTL related with the traits kernel weight and crop development, and while these were less correlated to yield than previous experience, they were highly heritable and relevant for southern environments. The phenology responses should largely be similar across seasons and less dependent on rainfall. Therefore, the selection of Compass alleles for developmental traits associated with grain yield in Chapter 4 is likely to be related to improved yield in seasons that are more typical. Nonetheless, as a result of 2016 conditions the population should be grown again under more typical Mediterranean environmental conditions to validate the findings in this study and from Chapter 4 or reveal new QTL controlling yield in this population.

The period of growth from awn primordia to tipping has been suggested as the most critical for determining grain number and extending the length of the phase using differing phenology genes could be promising for improving yield as it is directly related to spikelet survival and grain yield per main spike (Alqudah and Schnurbusch, 2014). However, the influence of the duration of the spikelet initiation phase has often been neglected in the literature. Among elite genotypes in Chapter 4, our experiments found no association between the length of the spike initiation phase and maximum spikelet primordia number and there was no association between maximum spikelet primordia and grains per spike, despite large genetic differences in both grains per spike and maximum spikelet primordia. This is important in the context of partitioning pre anthesis phases, as it would appear that shortening of the spike initiation phase might not compromise yield potential, either through reduced number of maximum spikelet primordia or grains per spike. Therefore, it may be possible for breeders to continue to shorten the period of time from DR – Awn Primordia. In Chapter 5, neither the Compass nor the Commander allele at QWn_2H that influenced inflorescence development was linked to spikelet length or number. The variant of PpD-H1 that decreased photoperiod sensitivity has previously been shown to increase the number of seeds per spike under favourable conditions (Digel *et al.*, 2015). While our study showed the photoperiod QTL had an effect on spike length, we also identified a QTL in ENV2 and ENV3 where the Compass allele lengthened the spike irrespective of the major Ppd 2H allele, that equates to 1-2 more grains per spike. This may help explain the improved yield of Compass. This is an important finding as it means breeders can manipulate the

number of grains per spike within contrasting photoperiod sensitivity groups, however this was only observed in the late May and June sowing time so needs to be validated in more environments.

The lack of significant relationships between phenology and spikelet survival in Chapter 4 suggests that factors other than phenology may be more important in determining spikelet survival. The number of spikes/m^2 were negatively correlated with spikelet survival suggesting that competition for resources or source-sink relationships during the critical period is an important trait rather than the length of time *per se*. The relationship between biomass, yield and grain numbers tends to support this idea and other mechanisms such as source-sink relationships and biomass partitioning may provide more scope for improved yield possibly during the immediate pre anthesis period and grain filling rather than phenology. Furthermore, QTL were identified in Chapter 5 that contributed to canopy architecture, NDVI and kernel weight along with QTL that contributed to spike length. These QTL could be further investigated to explain improvements in grain per spike and kernel weight of Compass as a result of improved resource allocation. Until a direct mechanistic link between pre-anthesis phenology traits and yield can be established, there appears to be limited opportunities for breeders to actively select for genotypes with variation in pre-anthesis patterns as a means to improve yield. Most pre-anthesis phases were strongly correlated with flowering time and as result, it is concluded that a dual focus on direct selection for an appropriate flowering time and yield remains one of the most effective approaches to optimise development patterns and the dynamics of grain yield. Furthermore, there was little merit in selecting for plasticity of phenology and yield traits. This is of importance in the context of the variable growing conditions experienced in Australia and Mediterranean environments as it validates the strategy currently adopted by breeders who have achieved a significant yield improvement in the cultivar Compass by modest to intermediate improvements in grain number without any trade-off in kernel grain weight.

Other considerations

Commercial plantings of barley in southern Australia are now moving towards early to mid-May planting dates. To our knowledge, the studies in Chapter 4 and Chapter 5 are among the first to identify developmental traits associated with yield from early May plantings. It has previously been suggested different development patterns may be required for early sowing (Young and Elliott, 1994). While Compass has been demonstrated to be higher yielding than Commander is in most environments of southern Australia in studies in 2014 and 2015, the 2016 data highlight additional complexities and that a delay in flowering time associated with increased photoperiod sensitivity (i.e. Commander) may be beneficial for April-early May sowing in favourable high yielding environments. Our results in 2014 and 2015 also revealed that the winter barley Urambie and other slow developing varieties might offer a more appropriate flowering date from early sowing in April

than the faster developing varieties in frost prone environments. However, in these studies in the absence of frost they either failed to achieve the same amount of biomass as the current well-adapted spring varieties or had a lower harvest index. While more research is needed, there is evidence that winter vernalisation alleles show promise (see Appendix 1). Compared to highly photoperiod sensitive ideotypes proposed by many researchers in the literature, the example of Compass and our own preliminary data outlined in Appendix 1 suggests it is possible to achieve similar flowering dates with different combinations of Ppd-H1 alleles and winter Vrn1-H1 and VrnH2 alleles. The yield performance or potential of these lines has never been explored in the context of early sowing or placed under any significant breeding selection for yield. The use of summer nurseries raises questions about the missed opportunity of other development alleles such as vernalisation, particularly as plants will still require a vernalisation period, which may only be partially fulfilled, or not likely to be experienced, in these selection environments. To avoid this, breeders could adopt more expensive double haploid systems or take care to ensure seedlings receive sufficient cold treatment to satisfy vernalisation requirements. Otherwise, the system will be selecting genotypes with bias towards low vernalisation requirements. The example of Compass highlights the potential limitations of selecting for early flowering in summer nurseries as there maybe alternative development patterns that could offer increased yield.

The results of this thesis provided some explanation for the yield benefits of Compass barley, but also raised a number of questions about crop development and yield development that require further investigation. The Commander x Compass population is an excellent resource to continue investigation in this area. Based on the result of these studies, the following recommendations are made:

Recommendations

- Selection for traits *per se* such as mean duration to anthesis and yield over multiple environments remains an effective strategy for continual yield improvement partly because adapted and high yielding varieties achieve high yields through multiple pathways.
- Further research should focus on spike initiation phase and its effect on the development of spikelet primordia. There may be scope to shorten this phase without compromising yield potential.
- The current studies focused on pre-anthesis phases and did not focus on the duration and timing of grain filling. Future studies should investigate this as a potential phase that describes the improved grain weight of Compass along with studies investigating the partitioning of assimilates such as water soluble carbohydrates prior and post anthesis to grain yield

- More evaluation for the Compass x Commander population in environments where yields are <6 tonne/ha is needed.
- Breeders can begin to select cultivars with reduced photoperiod sensitivity for adaptation to Australian environments.
- The five developmental QTL identified in this study may provide scope to further fine tune crop development under shorter photoperiods associated with autumn planting dates.
- Grain weight QTL on 4H and 6H should be tested in multiple environments and introgressed into material with higher grain numbers.
- Our study should inform improvements to current flowering time models such as APSIM. Not only should these effects be considered in future crop models, but also integrated with genomic data to investigate aspects of crop phenology that can be used for genetic dissection and the design of new ideotypes adapted to Australian environments.
- Breeders must adopt selection strategies to accommodate shifts in farming systems such as earlier planting dates, and reconsider the introgression of Vrn winter alleles into some faster developing spring cultivars as growers increasingly move their sowing dates forward.

Concluding remarks

The introduction of the European genetics (County) into Commander leading to the release of Compass has paved the way for a remarkable improvement in grain yield and adaptation. Major QTL for developmental traits were predominantly located near the candidate photoperiod response gene (Ppd-H1) on chromosome 2H. It was concluded that the faster development of Compass at May-June sowing dates was due to reduced responsiveness to photoperiod and its improved yield was associated with modest improvements in grain number and increased grain weight. Stable QTL for grain weight were found that breeders could exploit. Current breeding programs have historically focussed on developing photoperiod-responsive varieties, but the reduced photoperiod sensitivity of Compass suggests an alternative means of improving yield potential. This information will assist in developing more accurate flowering models and facilitate further fine-tuning of crop development and yield improvement under the short photoperiods associated with autumn planting dates in southern Australian environments. To our knowledge, this is the first information on the environmental modulation and genetic basis of phenology and yield related traits in elite cultivars conducted within the range of sowing dates over which barley is currently sown in the medium rainfall region of South Australia.

Alqudah, A. M. & Schnurbusch, T. (2014). Awn primordium to tipping is the most decisive developmental phase for spikelet survival in barley. *Functional Plant Biology* 41(4): 424-436.

Alzueta, I., Arisnabarreta, S., Abeledo, L. G. & Miralles, D. J. (2014). A simple model to predict phenology in malting barley based on cultivar thermo-photoperiodic response. *Computers and Electronics in Agriculture* 107: 8-19.

Boyd, W. J. R., Li, C. D., Grime, C. R., Cakir, M., Potipibool, S., Kaveeta, L., Men, S., Kamali, M. R. J., Barr, A. R., Moody, D. B., Lance, R. C. M., Logue, S. J., Raman, H. & Readj, B. J. (2003). Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. *Australian Journal of Agricultural Research* 54(11-12): 1277-1301.

Guo, L., Dai, J., Wang, M., Xu, J. & Luedeling, E. (2015). Responses of spring phenology in temperate zone trees to climate warming: A case study of apricot flowering in China. *Agricultural and Forest Meteorology* 201: 1-7.

Kernich, G. C., Halloran, G. M. & Flood, R. G. (1997). Variation in duration of pre-anthesis phases of development in barley (Hordeum vulgare). *Australian Journal of Agricultural Research* 48(1): 59-66.

Luedeling, E. & Gassner, A. (2012). Partial Least Squares Regression for analyzing walnut phenology in California. *Agricultural and Forest Meteorology* 158–159: 43-52.

Xu, Y. (2016). Envirotyping for deciphering environmental impacts on crop plants. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik* 129: 653-673.

Young, K. J. & Elliott, G. A. (1994). Art evaluation of barley accessions for adaptation to the cereal growing regions of western-australia, based on time to ear emergence. *Australian Journal of Agricultural Research* 45(1): 75-92.

Appendix 1: Is Vrn-H1 a missed opportunity for southern Australian barley growers?

Statement of Authorship

Title of Paper	Is Vrn-H1 a missed opportunity for southern Australian barley growers?
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Porker K, Coventry S, Trevaskis B, Fettell NA. 2017. Is Vrn-H1 a missed opportunity for southern Australian barley growers? Doing More with Less - Proceedings of the 18th Australian Society of Agronomy Conference, 24 – 28 September 2017. Ballarat, VIC, Australia: Australian Society of Agronomy.

Principal Author

Name of Principal Author (Candidate)	Kenton Porker		
Contribution to the Paper	Designed and conducted the time of sowing experiments at Roseworthy, conducted the analysis, wrote the paper.		
Overall percentage (%)	60%		
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	26 February 2018

Co-Author Contributions

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

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Neil Fettell		
Contribution to the Paper	Conducted the experiments in NSW, data interpretation and assisted in editing and reviewing manuscript		
Signature		Date	27 February 2018

Name of Co-Author	Ben Trevaskis

Contribution to the Paper	Developed germplasm, conducted genotyping, data interpretation		
Signature		Date	27 February 2018

Name of Co-Author	Stewart Coventry		
Contribution to the Paper	Data interpretation, editing and reviewing manuscript		
Signature		Date	28TH FEBRUARY. 2018

Please cut and paste additional co-author panels here as required.

Is Vrn-H1 a missed opportunity for southern Australian barley growers?

Kenton Porker[1], Stewart Coventry[1], Ben Trevaskis[2] and Neil Fettell[3]

1 School of Agriculture Food and Wine, The University of Adelaide Glen Osmond, SA 5064, Kenton.porker@sa.gov.au
2 CSIRO Agriculture, GPO Box 1600 Canberra, ACT 2601
3 Central West Farming Systems and University of New England, PO Box 171 Condobolin, NSW 2877

Abstract
Over the last decade, there has been a trend to earlier sowing of cereals. Growers are seeking varieties that develop slower to match needs of minimising reproductive frost risk, avoid high grain-filling temperatures and terminal water stress. Historically barley breeders focused on developing cultivars with a short mean duration to flowering through direct and indirect selection of photoperiod sensitivity (*Ppd*) alleles and insensitive vernalisation (*Vrn*) alleles. This paper discusses the concept that the lack of winter *Vrn-H1* alleles in Australian cultivars may be a missed opportunity for southern Australian barley growers and presents the history of winter barleys in Australia, and the merits of re-introducing winter *Vrn* alleles into breeding programs. Based on preliminary data it is possible to achieve a similar flowering date, and competitive yields with different combinations of phenology genes including winter *Vrn* alleles from earlier sowing.

Keywords
Phenology, sowing time, vernalisation, photoperiod.

Introduction

Matching crop phenology with availability of resources and avoiding stress events during flowering and grain-filling are key factors influencing yield and crop adaptation (Richards 1991). In Australia, climatic conditions in the temperate cereal production areas define the periods for sowing and the phenological events, which follow, such as the transition from the vegetative to reproductive phase and flowering time. Various genes control the timing and duration of developmental phases, mainly attributable to photoperiod (*Ppd*), low temperature vernalisation (*Vrn*) response genes, and earliness *per se* (*Eps or Eam*) loci (Campoli and von Korff 2014). Yield improvements have been achieved through direct selection of yield based on traditional May sowing dates and an appropriate flowering time, resulting in indirect selection for phenology gene combinations favouring this farming system environment. The recent decade trend of earlier sowing dates using current varieties could result in undesirable early flowering and slower developing varieties with new developmental gene combinations may be necessary to fit this farming system. This paper aims to discuss the merits of utilising *Vrn* alleles from winter barleys to improve yield in early sown crops.

What is a winter barley?

Vernalisation is the requirement for a period of exposure to low temperature before the plant apical meristem will transition from vegetative to reproductive development. Vernalisation alters the length of the vegetative phase, and hence floral initiation, which indirectly affects the duration of subsequent pre-heading phases.
Genotypes differ in low temperature requirement in the duration and intensity of effective vernalising temperatures from no requirement in "traditional spring types" to 3-12˚C for an extended period in winter types (Garcia del Moral et al. 2002). The winter type is predominantly controlled by the three vernalisation genes, *Vrn-H1, Vrn-H2*, and *Vrn-H3*. Different *Vrn-H1* genes are the primary driver of vernalisation response (Trevaskis et al. 2007), and interact with *Vrn-H2* and *Vrn-H3* vernalisation genes and the photoperiod pathway. Unlike spring barley, true winter types require adequate cold stimulus for the *Vrn-H1* gene to induce development of the reproductive meristem, and *Vrn-H2* overrides *Ppd-H1* (photoperiod response) if exposed to sufficiently low temperatures (Distelfeld et al. 2009). Spring barleys do not have a recognised vernalisation requirement associated with *Vrn-H1* and *Vrn-H2* so flowering is dependent on photoperiod (*Ppd-H1*) and *earliness per se* genes.

Breeding and evaluating winter barleys

The first 'modern' winter barley in Australia was Ulandra, selected and released in 1987 by NSW DPI (Read and Macdonald 1987) followed by Urambie in 2005, a semi-dwarf feed barley aimed at both dual purpose and grain only situations suited to early March to mid-May sowing in NSW. Prior to this, most adapted Australian cultivars and spring types introduced from Europe, Canada, and Japan have either no or a minimal

vernalisation requirement (Boyd et al. 2003). A study revealed there is limited variation in *Vrn-H1* in spring cultivars grown in southern Australia, and the majority have a deletion of the winter type at *Vrn-H2* (Porker unpublished). Boyd et al. 2003 concluded that the best adapted barley cultivars for Australian low-medium rainfall environments are early-maturing spring types that combine a short vegetative phase, exhibit a high photoperiod response and limited vernalisation requirement. This plant ideotype was easy to select in summer selection nurseries utilised by Australian breeding programs, based on selection for early flowering and limited tillering under long hot days. However, this traditional ideotype breeding approach is challenged by the knowledge that a recent release, Compass, carries a photoperiod insensitive allele, but has achieved a short mean duration to flower in winter plantings by combining other developmental genes. It is therefore possible to achieve desired flowering dates with phenology gene combinations other than those previously explored. True winter types sown in summer selection nurseries fail to flower and produce viable seed, meaning any lines possessing winter *Vrn-H1* alleles do not progress to yield evaluation trials. The lack of introgression of the winter *Vrn-H1* allele may be a missed opportunity for southern Australian barley growers.

The case for winter barleys?

Early sowing evaluation trials were conducted on the Southern Coast of WA by (Portmann and Young 1987), as they recognised that "suitable material was not being generated out of the traditional germplasm being used in the program. Our attention then started to turn to vernalisation responsive barleys." Winter lines showed promise for early sowing in WA but were deemed unsuitable for malting (Table 1); relative to Schooner they were either similar or higher yielding. They noted the slow early vigour of winter types and reduced dry matter during winter compared to spring lines and concluded the task confronting breeders was to select less temperature sensitive winter types that could maintain growth rates similar to spring types.

Table 1. Yields (t/ha) of selected spring and winter barley lines in 1986 time of sowing trials (Portmann and Young 1987).

Line	Early May Sowing (Mt Barker 1986)	April Sowing (Esperance 1986)
Stirling	3.97	1.25
Schooner	4.86	1.52
WU35 (Winter)	5.00	2.01
WU44 (Winter)	5.11	3.10
l.s.d. (5%)	0.49	0.44

The NVT trials were interrogated for data on winter release, Urambie. Urambie has been included in 131 NVT trials across QLD, NSW and Vic but not included in South Australian NVT trials. The sowing dates have arguably been too late for adequate evaluation of Urambie. Few trials have been sown before the first week of May, in the ideal window for a winter barley, with mean sowing dates trending beyond the last week of May across regions (Table 2). Despite this, Urambie has performed close to site mean yield across many sites and seasons. Analysis of Urambie performance versus sowing dates revealed little evidence of any sowing time interaction (data not presented) suggesting trials were simply sown too late. Although late May sowing dates quickly saturate vernalisation requirements, highly photoperiod sensitive cultivars have been favoured in Australia due to reduced tillering and improved grain weight compared to Urambie.

Table 2. Trial number, earliest, latest and mean sowing dates, average Urambie yield (t/ha) and percentage of site mean yield for 131 NVT trials over 12 seasons in 7 barley growing regions (NVT online).

State	Region	No. trials	Earliest sow date	Latest sow date	Mean sow date	Urambie Av. yield	Percentage of SMY
NSW	N/E	26	12-May	5-Jul	3-Jun	3.75	96
NSW	N/W	39	10-May	6-Jul	27-May	3.44	98
NSW	S/E	17	13-May	5-Jul	28-May	4.08	101
NSW	S/W	29	10-May	10-Jul	24-May	3.51	98
QLD	SEQ	2	7-Jun	7-Jun	7-Jun	4.35	106
QLD	SWQ	2	24-May	1-Jun	28-May	5.23	107
Vic	S/W	16	5-May	30-May	15-May	5.28	96

Methods

Dr Ben Trevaskis at CSIRO developed barley near isogenic lines (NILs) with variation in vernalisation requirement and/or photoperiod sensitivity. Preliminary yield trials were conducted on five NILs, Commander, Compass, and Urambie in 2016 at Roseworthy and Condobolin. The NILs contained different

combinations of *Vrn-H1*, *Vrn-H2*, and *Ppd-H1* development genes backcrossed to the ultra- early barley genotype WI4441, representing different facultative, winter, and spring molecular ideotypes. The NILs *B01* and *B02* were winter types with different photoperiod sensitivity genes. Whereas other lines were either spring or facultative types combining differences in photoperiod sensitivity (Table 3). Lines were sown on 5th May at Roseworthy and 28th April at Condobolin in replicated field plots. Anthesis dates were recorded at both sites and at Condobolin, dissections were used to identify double ridge and awn primordia stages.

Table 3. The near isogenic lines and varieties used in field trials at Roseworthy and Condobolin in 2016, showing their major development alleles growth habit (Vrn S = Spring allele, W = Winter alleles, Photoperiod I = reduced sensitivity to day length, S = Increased sensitivity to daylength).

LINE	*Vrn-H1*	*VRN-H2*	*Photoperiod*	Barley type
B06	S	W	I	Facultative
B15	S	S	S	Spring
Compass	S	S	I	Spring
B10	S	S	S	Spring
Commander	S	S	S	Spring
B01	W	W	S	Winter
B02	W	W	I	Winter
Urambie	W	W	I	Winter

Results

At Roseworthy the winter line *B02* flowered seven days later than Commander, at Condobolin the difference was four days (Tables 4 and 5). The effect of photoperiod was evident by delayed flowering of Commander compared to Compass. It was possible to achieve a flowering date similar to or earlier than the traditional Commander type in both environments with a phenology gene combination that was previously considered unsuitable. Despite not being selected for yield, the NILs were competitive in both environments and at Roseworthy *B02* was the highest yielding line. Urambie was equal highest yielding at Condobolin. This shows the potential for utilising *Vrn-H1* winter alleles to improve yield in early sowing environments. Winter lines had a longer period to double ridge (Figure 1) and higher spike numbers. Spring lines were quickest to double ridge. The winter lines differed in time to double ridge and from awn primordia to anthesis, indicating diversity of development patterns that could be exploited within winter types. A common feature of winter lines has been a low harvest index and grain weights. However, in these trials there is little evidence to suggest a lower harvest index compared to springs although grain weights were noticeably lower in B02 at both sites (Tables 4 and 5) which may have implications for small grain screenings.

Table 4. Anthesis date, yield, harvest index, kernel weight, and ear numbers at Roseworthy 2016, sown May 5.

Genotype	Anthesis date	Yield (t/ha)	HI	K Wt (mg)	Ears/m2
B06	25-Aug	7.36	0.34	40.92	843
B15	28-Aug	6.66	0.30	44.76	519
Compass	24-Aug	7.66	0.36	49.02	578
B10	2-Sep	7.25	0.31	41.02	617
Commander	4-Sep	6.79	0.34	44.47	509
B01	26-Aug	7.48	0.42	44.52	869
B02	11-Sep	8.25	0.39	42.03	892
F pr.		<.001	0.01	<.001	<.001
l.s.d.		0.48	0.032	1.12	124

Conclusion

While more research is needed, based on traditional May – June sowing dates and the limited cultivar data there is evidence that winter vernalisation alleles show promise. Compared to highly photoperiod sensitive ideotypes proposed by many researchers in the literature, our own preliminary data suggests it is possible to achieve similar flowering dates with different combinations of *Ppd-H1* alleles and winter *Vrn1-H1* and *Vrn-H2* alleles. The yield performance or potential of these lines has never been explored in the context of early sowing or placed under any significant breeding selection for yield. It is understandable why there has been limited selection within breeding programs, given their use of summer nurseries, glasshouse or growth room environments to speed up and select early generations. However, this also has implications for phenology, as plants will still require a vernalisation period that may only be partially fulfilled or not likely to be

experienced in these selection environments. To avoid this, breeders could adopt more expensive double haploid systems or take care to ensure seedlings receive sufficient cold treatment to satisfy vernalisation requirements. Otherwise, the system will be selecting genotypes with bias towards lower vernalisation requirements. We believe the time is right to reconsider the introgression of *Vrn* winter alleles into some faster developing spring cultivars as growers increasingly move their sowing dates forward.

Table 5. Anthesis date, yield, harvest index, kernel weight, and ear numbers at Condobolin 2016, sown 28 April.

Genotype	Anthesis date	Yield (t/ha)	HI	K Wt (mg)	Ears/m2
B06	18-Aug	4.89	0.33	45.5	568
B15	18-Aug	5.12	0.34	46.2	543
Compass	19-Aug	4.68	0.34	53.8	462
B10	24-Aug	4.28	0.33	42.5	601
Commander	3-Sep	4.38	0.32	41.8	574
B01	4-Sep	3.74	0.33	42.3	754
B02	4-Sep	3.95	0.32	40.7	846
Urambie	7-Sep	4.94	0.33	43.8	598
F pr.		<.001	<.001	<.001	<.001
l.s.d.		0.53	0.03	3.1	129

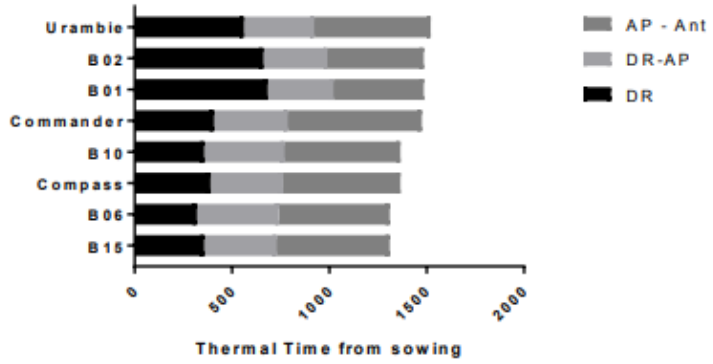


Figure 1. Phase lengths in thermal time (^{0}C days) for eight barley line sown on 28 April at Condobolin 2016. The phases are sowing to double ridge (DR), double ridge to awn primordia (AP), awn primordia to anthesis (Ant).

References

Boyd WJR, et al. (2003). Conventional and molecular genetic analysis of factors contributing to variation in the timing of heading among spring barley (Hordeum vulgare L.) genotypes grown over a mild winter growing season. Australian Journal of Agricultural Research 54 (11-12), 1277-1301.

Campoli C and von Korff M (2014).Chapter Five - Genetic Control of Reproductive Development in Temperate Cereals. In: Advances in Botanical Research 72, 131-158. F Fabio Ed. Academic Press.

Distelfeld A, Li C and Dubcovsky J (2009). Regulation of flowering in temperate cereals. Current Opinion in Plant Biology 12 (2), 178-184.

Garcia del Moral LG, Miralles DJ and Slafer GA (2002).Initiation and appearance of vegetative and reproductive structures throughout barley development. In: Barley science: recent advances from molecular biology to agronomy of yield and quality, pp. 243-267.

Portmann PA and Young KF (1987). The Role of Winter Barley in Western Australia. In: Proceedings Australian Barley Technical Symposium, 11-15th October 1987. Wagga Wagga NSW.

Read BJ and Macdonald AF (1987). Ulandra--the first winter barley for Australia. In: Proceedings Australian Barley Technical Symposium, 11-15th October 1987. Wagga Wagga NSW.

Richards RA (1991). Crop improvement for temperate Australia - future opportunities. Field Crops Research 26(2), 141-169.

Trevaskis B, Hemming MN, Dennis ES and Peacock WJ (2007). The molecular basis of vernalisation-induced flowering in cereals. Trends in Plant Science 12 (8), 352-357.