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ORIGINAL ARTICLE

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

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Abstract
Key message **Study of three interconnected populations identified 13 maturity QTL of which eight collocate with phenology genes, and 18 QTL for traits associated with adaptation to drought-prone environments.**
Abstract QTL for maturity and other adaptive traits affecting barley adaptation were mapped in a drought-prone environment. Three interconnected doubled haploid (DH) populations were developed from inter-crossing three Australian elite genotypes (Commander, Fleet and WI4304). High-density genetic maps were constructed using genotyping by sequencing and single nucleotide polymorphisms (SNP) for major phenology genes controlling photoperiod response and vernalization requirement. Field trials were conducted on the three DH populations in six environments at three sites in southern Australia and over two cropping seasons. Phenotypic evaluations were done for maturity, early vigour, normalized difference vegetation index (NDVI) and leaf chlorophyll content (SPAD), leaf waxiness and leaf rolling. Thirteen maturity QTL were identified, all with significant QTL × environment interaction with one exception. Eighteen QTL were detected for other adaptive traits across the three populations, including three QTL for leaf rolling, six for leaf waxiness, three for early vigour, four for NDVI, and two QTL for SPAD. The three interlinked populations with high-density linkage maps described in this study are a significant resource for examining the genetic basis for barley adaptation in low-to-medium rainfall Mediterranean type environments.

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Introduction

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

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

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

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

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

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

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

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

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

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

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

Materials and methods

Plant materials

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

Table 1 Description of trial environments

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

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

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

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

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

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

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

Field trials

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

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

Phenotyping

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

Genotyping and linkage map construction

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

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

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

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

Statistical analysis

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

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

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

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

QTL analysis

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

Results

Variation in plant development

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

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

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

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

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

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

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

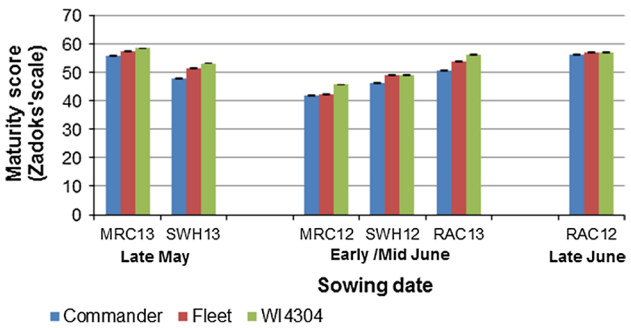

Fig. 1 Variation in maturity scores between the parents at different sowing dates

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

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

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

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

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

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

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

[c] This QTL showed no QTL × environment interaction

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

Genetic analysis

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

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

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

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

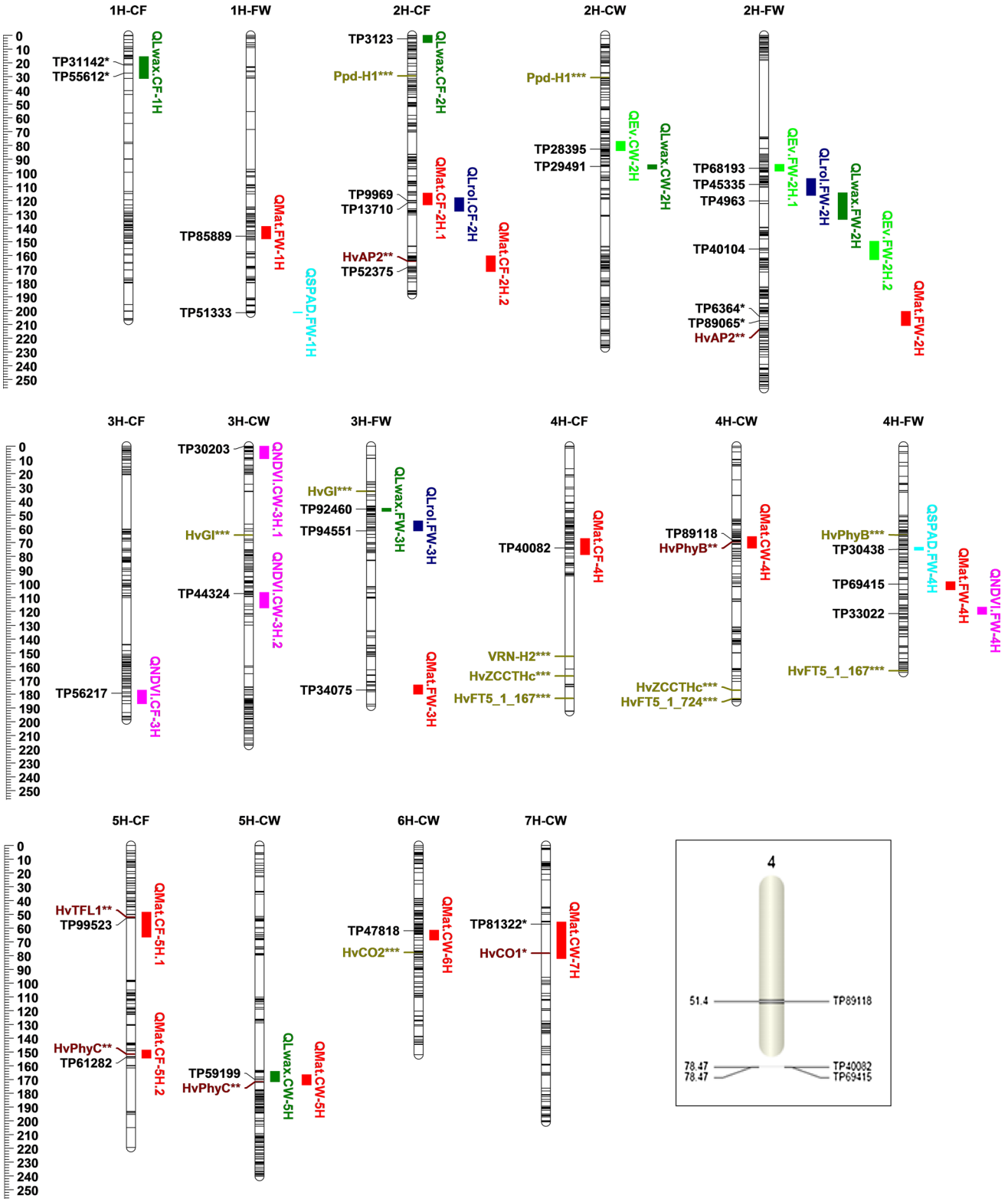

Fig. 2 QTL positions for maturity and other developmental traits in the CF, CW and FW DH populations. The vertical QTL bars represent the 1.5-LOD QTL interval. Only significant markers at the QTL peak are shown on the maps. *Asterisk* indicates the closest markers from both sides of the QTL peak are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

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

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

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

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

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

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

Discussion

QTL for adaptive traits to drought-prone environments

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

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

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

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

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

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

Maturity QTL

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

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

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

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

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

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

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

QMat.FW-3H seems to be related to the maturity genes *eps3L/eam10*, which co-located with the *HvLUX* gene at the proximal end of chromosome 3H. *Eam10* causes circadian defects and interacts with *Ppd-H1* to accelerate flowering under long and short days. Maturity QTL in this region has also been reported in other populations including Alexis × Sloop, Halcyon × Sloop, Tallon × Kaputar, and Arapiles × Franklin (Boyd et al. 2003).

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

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

Conclusions

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

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

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

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Compliance with ethical standards

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

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