Agronomy **2015**, *5*, 152-179; doi:10.3390/agronomy5020152

OPEN ACCESS

agronomy

ISSN 2073-4395

www.mdpi.com/journal/agronomy

Article

Genomic Regions for Embryo Size and Early Vigour in Multiple Wheat (*Triticum aestivum* L.) Populations

Chris Moore [1] and Greg Rebetzke [2,*]

[1] Department of Plant and Food Sciences, The University of Sydney, NSW 2006 Australia now Intergrain Wheats Pty Ltd, 19 Ambitious Link, Bibra Lake WA-6162, Australia; E-Mail: CMoore@intergrain.com

[2] CSIRO Agriculture Flagship, P.O. Box 1600, Canberra ACT-2601, Australia

* Author to whom correspondence should be addressed; E-Mail: Greg.Rebetzke@csiro.au; Tel.: +61-2-6246-5153; Fax: +61-2-6246-5399.

Academic Editor: Bertrand Hirel

Received: 22 January 2015 / Accepted: 23 April 2015 / Published: 5 May 2015

Abstract: Greater early vigour has potential for increasing biomass and grain yields of wheat crops in Mediterranean-type environments. Embryo size is an important determinant of early vigour in barley and likely to contribute to greater vigour in wheat. Little is known of the underlying genetic control for embryo size, or its genetic association with early vigour in wheat. Over 150 doubled-haploid lines in each of three unrelated wheat populations varying for embryo size and early vigour were phenotyped across multiple controlled environments. The Quantitative Trait Locus (QTL) mapping was then undertaken to understand genetic control and chromosomal location of these characteristics. Genotypic variance was large and repeatable for embryo and leaf size (width and length) but not specific leaf area or coleoptile tiller size. Genetic correlations for embryo size with leaf width and area were moderate to strong in size while repeatabilities for embryo size and early vigour were high on a line-mean basis. Multiple genomic regions were identified of commonly small genetic effect for each trait with many of these regions being common across populations. Further, collocation of regions for many traits inferred a common genetic basis for many of these traits. Chromosomes 1B, 5B, 7A and 7D, and the *Rht-B1b* and *Rht-D1b*-containing chromosomes 4B and 4D contained QTL for embryo size and leaf width. These studies indicate that while early vigour is a genetically complex trait, the selection of larger embryo progeny can be readily achieved in a wheat breeding program targeting development of high vigour lines.

Keywords: heritability; weed competitiveness; establishment; nutrient uptake; water use; selection

1. Introduction

Early vigour has been widely reported to provide improved productivity of wheat in semi-arid environments, and particularly those environments in which rainfall patterns are Mediterranean in nature [1–4]. Large areas of the Australian wheat production environment, particularly in the southern and western cropping regions, are of a Mediterranean nature. Early vigour has been shown to increase wheat productivity through increased biomass [2–5], and similar increases have been reported in other crops such as barley [6–8]. Early vigour has the potential to increase crop water-use efficiency in moisture-limited environments [9,10].

Breeding opportunities for increased water-use efficiency have been previously defined [11] as: (1) increased crop use of available water; (2) increased biomass per unit of water utilised by the crop; and (3) increased harvested product. The effect of greater biomass in the early growth of the crop is likely to increase soil shading thereby reducing soil moisture evaporation and retaining more moisture for crop growth [12]. The increased radiation interception is likely also important for improving the ability of the crop to compete with weeds [13,14]. This potential has also been observed in other crops such as barley [8] and rice [15]. There is also potential for high vigour wheats in maintaining biomass and grain yield with late sowing.

Embryo size is correlated with seed size in wheat [6,16–19]. Seed size has also been reported to influence early vigour and seedling characteristics in wheat [6,20–22] and other species including barley [23], rice [24], durum wheat [25], triticale [26] and maize [27]. The close relationship between seed size and early vigour characteristics highlights the importance of accounting for such effects when attempting genetic analyses of early vigour traits. Further, it has been previously reported [28] that the correlation of seed size to early vigour performance might be more pronounced under conditions of moisture stress than in well-watered conditions.

Genomic regions and underlying genetic loci associated with early vigour have been reported for wheat by several sources [29–33]. However, to date there have been no published reports describing genes or Quantitative Trait Locus (QTL) for embryo size in wheat. That aside, genetic studies of embryo size were reported in other cereal species and particularly rice (e.g., [24,34]).

This paper aims to characterise embryo size and early vigour for three doubled-haploid wheat populations in order to assess genetic variation and covariation, and inheritance for these characteristics. QTL analyses and mapping was then conducted to establish chromosomal regions of importance and their robustness for these traits across populations.

2. Results

Assessment of embryo size and early vigour will be reported on a population basis. The QTL analyses on the Sunco/Tasman and Cranbrook/Halberd populations will then be discussed separately.

2.1. Phenotypic Characterisation

2.1.1. Sunco/Tasman Population

The Sunco/Tasman DH population contained significant genotypic variation for embryo characteristics width, length and derived-area (Table 1 and Figure 1). The range in genotype means was 72%, 90% and 134% for embryo length, width and area, respectively. The population mean for embryo width (1.37 mm) was approximately equal and not statistically different ($p > 0.05$) to the mid-parent value (1.35 mm). Similarly, the mid-parent and population means were not statistically different for embryo length (2.31 *vs.* 2.32 mm) and embryo area (2.27 *vs.* 2.31 mm^2). Frequency distributions for embryo traits in the Sunco/Tasman population are illustrated in Figure 1 with progeny means considered to be normally distributed for all three traits. Although the population means were relatively similar to the mid-parent value for each trait, many lines produced values considerably in excess or less than the parent values suggesting transgressive segregation. The observed transgressive segregation could also reflect non-random sampling of genotypes or alleles (disequilibrium) although this is unlikely in these larger populations.

Frequency distributions for early vigour (above-ground plant) traits are given in Figure 2. Genotypic differences were significant for all traits except primary tiller length (Table 1). For all traits genotype means were assumed to be normally distributed with the exception of coleoptile tiller length (size). For coleoptile tiller size, many lines did not produce a coleoptile tiller for one of two reasons: (1) lacked genotypic potential; or (2) timing of the vigour harvest did not allow for tiller late expression. Interestingly for this population this phenomenon was not exhibited for primary tiller length with many of the DH lines producing a primary tiller prior to the early vigour harvest. For most early vigour traits the population mean was found to be approximately equivalent to the mid-parent mean. The exception here was primary tiller length where the population mean was similar in size to the high-parent mean and not the mid-parent value. Considerable transgressive segregation was evident for all early vigour traits in this population.

Variance components from the analysis of embryo traits are given in Table 1. Seed weight was found to be a significant covariate for all traits and so was included in the combined analysis. Increases in seed size were commonly associated linearly with increases in trait value (data not shown). Genotypic variance was large and statistically significant for embryo width, length and area. Genotypic × environment interactions were found to be significant but relatively small in size for all three embryo traits. Narrow-sense heritabilities were greatest on a line-mean basis for embryo length (78%) and smallest for embryo width (59%). Heritabilities were also moderate in size for embryo traits on a single-seed basis (Table 1).

Table 1. Variance components (σ^2 ± standard errors) and narrow-sense heritability (on a line-mean and single-plant basis; ±standard error) for multi-environment assessment of early vigour parameters for the Sunco/Tasman doubled-haploid bread wheat population. The statistical significance of seed weight as a covariate for each trait analysis is also given.

Parameter	Embryo Width [A]	Embryo Length [A]	Embryo Area [A]	Leaf 1 Width [A]	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area [A]	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
σ^2_G	0.255 ± 0.05 **	1.29 ± 0.18 **	2.42 ± 0.35 **	2.12 ± 0.39 **	13.3 ± 3.2 **	3347 ± 600 **	125.2 ± 19.7 **	49 ± 15 **	48 ± 18 **	22 ± 16 ns	401 ± 117 **
$\sigma^2_{G\times E}$	0.011 ± 0.07 *	0.05 ± 0.01 **	0.12 ± 0.05 **	0.27 ± 0.32 **	6.9 ± 3.3 *	1110 ± 473 **	24.6 ± 12.8 *	32 ± 17 *	69 ± 24 **	61 ± 24 **	472 ± 128 **
$\sigma^2_{residual}$	0.349 ± 0.04 **	0.61 ± 0.06 **	1.54 ± 0.19 **	4.83 ± 0.36 **	40.6 ± 2.9 **	5444 ± 393 **	152.3 ± 11.4 **	218 ± 16 **	225 ± 18 **	264 ± 21 **	1380 ± 102 **
$h^2_{line\text{-}mean}$	0.59 ± 0.10 **	0.78 ± 0.09 **	0.73 ± 0.10 **	0.78 ± 0.08 **	0.56 ± 0.08 **	0.70 ± 0.09 **	0.77 ± 0.10 **	0.48 ± 0.07 **	0.40 ± 0.08 **	0.23 ± 0.11 *	0.46 ± 0.08 **
$h^2_{single\text{-}plant}$	0.42 ± 0.06 **	0.66 ± 0.07 **	0.59 ± 0.10 **	0.44 ± 0.06 **	0.22 ± 0.04 **	0.34 ± 0.05 **	0.41 ± 0.4 **	0.16 ± 0.05 *	0.14 ± 0.04 *	0.06 ± 0.04 ns	0.18 ± 0.05 **
Seed weight	*	**	**	**	*	**	**	*	ns	ns	**

*, ** Indicates statistical significance at $p = 0.05$ and 0.01, respectively; ns: non-significant at $p = 0.05$; [A] variance component × 100.

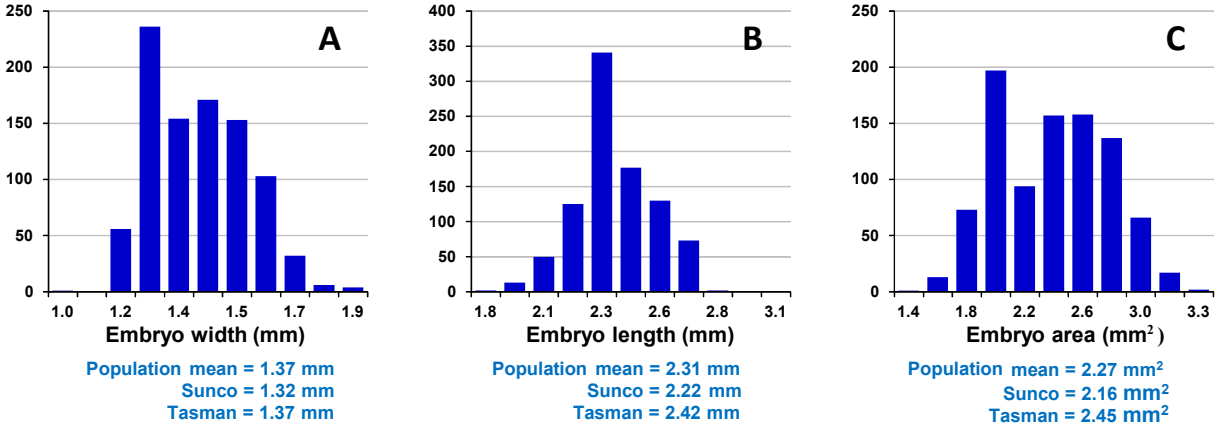

Figure 1. Frequency distributions of embryo characteristics for the Sunco/Tasman doubled-haploid population. Traits shown are (**A**) embryo width; (**B**) embryo length; and (**C**) embryo area. Population and parental means are given beneath chart.

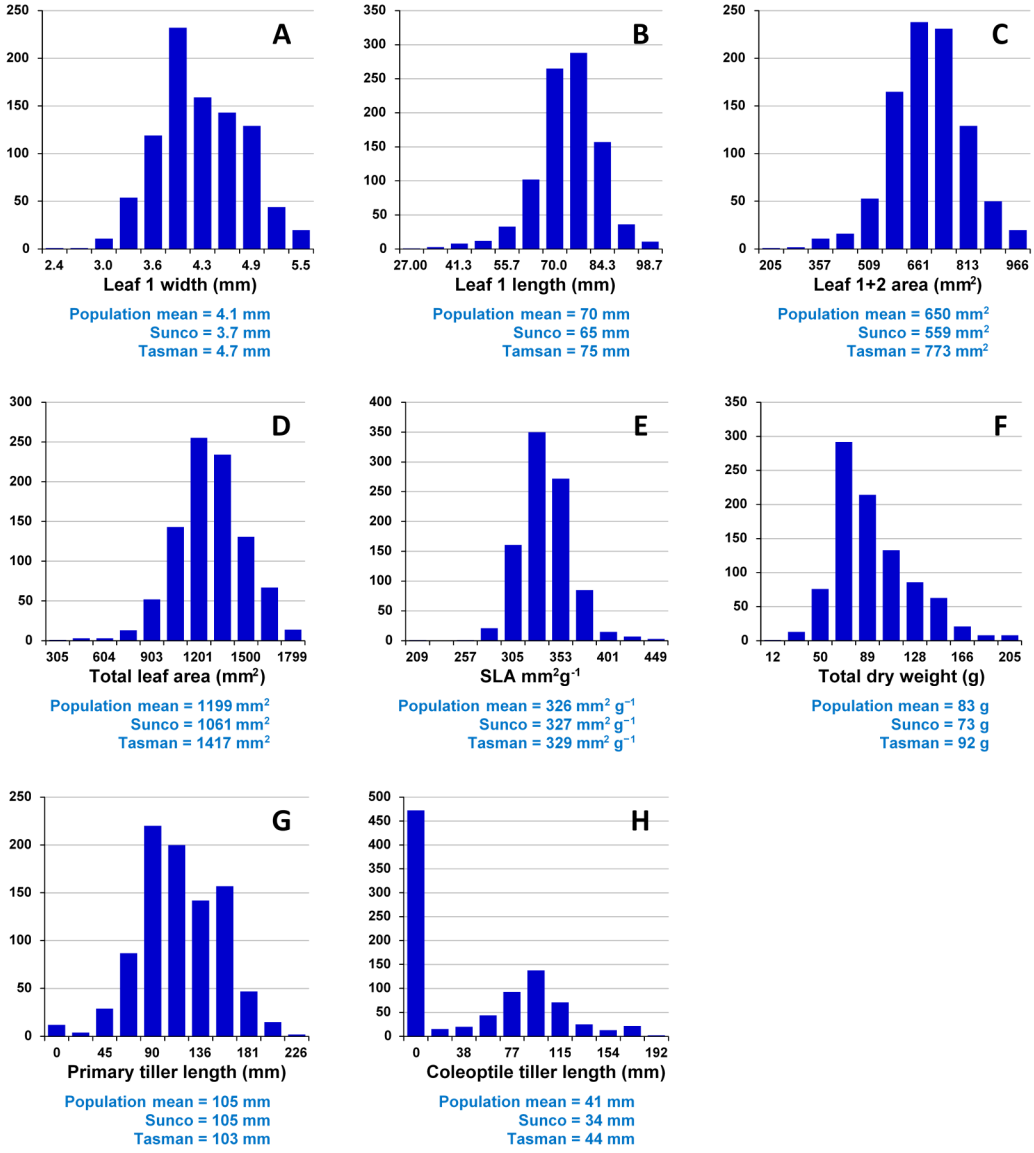

Figure 2. Frequency distributions of various early vigour traits for the Sunco/Tasman doubled-haploid bread wheat population. Traits shown are (**A**) Leaf 1 width; (**B**) Leaf 1 length; (**C**) Leaf 1+2 area; (**D**) Total leaf area; (**E**) Specific Leaf Area (SLA); (**F**) Total dry weight; (**G**) Primary tiller length; and (**H**) Coleoptile tiller length. Population and parental means are also given.

Variance components for early vigour traits in the Sunco/Tasman population are given in Table 1. Seed weights were found to be significant in the analysis of most traits and were treated as covariates as required. In all traits with the exception of primary tiller length, genetic variances were large and statistically significant. No significant genetic variance was observed for primary tiller number in this population. The magnitude of genotypic × environment interactions were variable across traits with those for specific leaf area, total plant dry weight, primary tiller and coleoptile tiller lengths being large and statistically significant. This was reflected in the narrow-sense heritabilities (line-mean) for these traits being small (23%–48%). Leaf one width and total leaf area exhibited the largest line-mean narrow-sense heritabilities of 78 and 77%, respectively.

Genetic correlations for embryo dimensions with early vigour traits are listed for the Sunco/Tasman population in Table 2. In all covariance analyses seed size was included in the full statistical model. There was a strong and significant correlation of embryo area with both embryo width (r_g = 0.88 **) and length *(*r_g = 0.84 **). The genetic relationship of embryo length with width was also significant and positive albeit the correlation was smaller (Table 2). The relationship for embryo traits with genotypic variation in early vigour were found to be largely positive although for some traits were not statistically different from zero. The largest genetic correlations with embryo traits were for embryo length and specific leaf area (r_g = 0.58 **); embryo area and total dry weight (r_g = 0.47 **); and embryo width and leaf 1 width (r_g = 0.44 **).

Generally, the relationships for embryo size with leaf 1 and coleoptile tiller length were not significant, or small. The relationship for embryo size with primary tiller length was evident as embryo width and length were not in themselves significant correlated with tiller length whereas embryo area was correlated with tiller length. The genetic correlation for leaf 1 width and total leaf area was strong and significant (r_g = 0.83 **) while total leaf area and plant dry weight were themselves strongly correlated (r_g = 0.92 **) (data not shown).

Table 2. Genetic correlations (r_g) for the embryo characteristics and early vigour parameters of the Sunco/Tasman doubled-haploid bread wheat population.

r_g	Embryo Width	Embryo Length	Embryo Area	Leaf 1 Width	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
Embryo width	-	0.56 **	0.88 **	0.44 **	0.09 ns	0.26 **	0.34 **	0.26 **	0.27 **	−0.13 ns	0.19 *
Embryo length	-	-	0.84 **	0.33 **	0.09 ns	0.25 **	0.33 **	0.58 **	0.32 **	0.03 ns	0.21 *
Embryo area	-	-	-	0.36 **	0.12 ns	0.34 **	0.41 **	0.36 **	0.47 **	0.26 **	0.15 ns

*, ** Indicates statistical significance at p = 0.05 and 0.01, respectively; ns: non-significant at p = 0.05.

2.1.2. Cranbrook/Halberd Population

As was the case for the Sunco/Tasman population, the Cranbrook/Halberd population exhibited large and statistically significant range in progeny means for embryo width, length and area (Figure 3). The range was smaller for embryo width (0.9 mm) and length (1.3 mm) than for embryo area (2.11 mm^2). For embryo width, the population mean (1.43 mm) was consistent with the large width parent Halberd (1.42 mm), and was statistically larger than the mid-parent value of 1.41mm. Embryo area was similar to embryo width in this respect, as the population mean (2.51 mm^2) was statistically larger than the mid-parent value (2.46 mm^2) and larger than the mean for the larger embryo area parent Halberd (2.49 mm^2). Both parents shared similar estimates for embryo length (2.41 mm), and these were similar in size to the population mean of 2.44 mm.

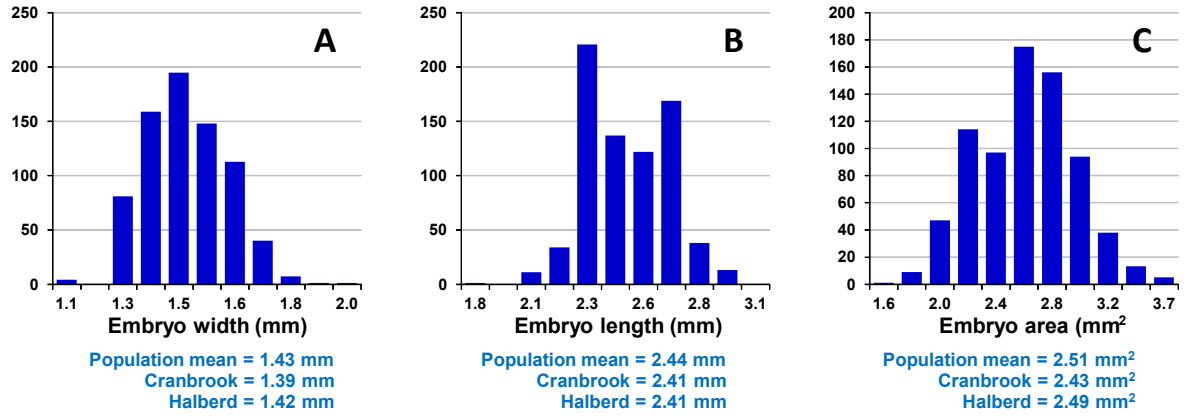

Figure 3. Frequency distributions of embryo characteristics for the Cranbrook/Halberd doubled-haploid bread wheat population. Traits shown are (**A**) Embryo width; (**B**) Embryo length; and (**C**) Embryo area. Population and parental means are also given.

Frequency distributions for progeny means for the embryo size traits are given in Figure 3. Progeny distributions for all three embryo traits was assumed to be Gaussian in nature, and as for the Sunco/Tasman population, genotypic variation exceeding either parent was observed for each embryo trait in the Cranbrook/Halberd population. Early vigour frequency distributions for Cranbrook/Halberd are given in Figure 4. As was the case with Sunco/Tasman, each of the Cranbrook/Halberd traits was found to be normally distributed except for coleoptile tiller length. The population mean for leaf one width was consistent in size with the low parent (Cranbrook) whereas for specific leaf area, the population mean exceeded both mid-parent and parental means. For all other traits, the population mean was approximately equal in size with the mid-parent mean. Considerable transgressive segregation among progeny means was evident for all early vigour traits for this population.

The variance components for embryo traits are given in Table 3. Variation in seed weight was found to be statistically significant for all three dimension traits and was included in the analysis as a covariate. Genotypic variances were found to be large and statistically significant for all traits whereas genotype × environment interactions although significant were commonly relatively small in size. This is reflected in the very high narrow-sense heritabilities (line-mean) observed for this population, with embryo width estimated at 87%, and both embryo length and embryo area estimated at 90%. The high heritabilities for embryo traits were the highest recorded for any population in this study (*cf.* Table 1).

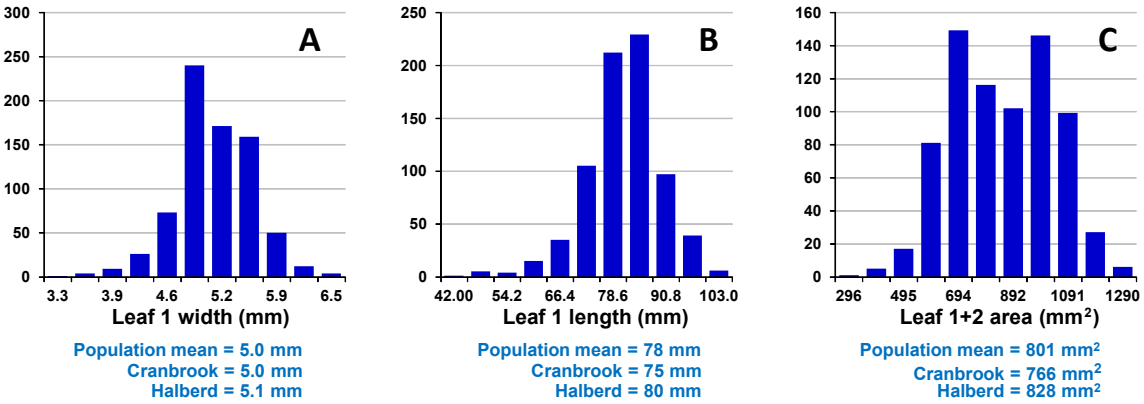

Figure 4. *Cont.*

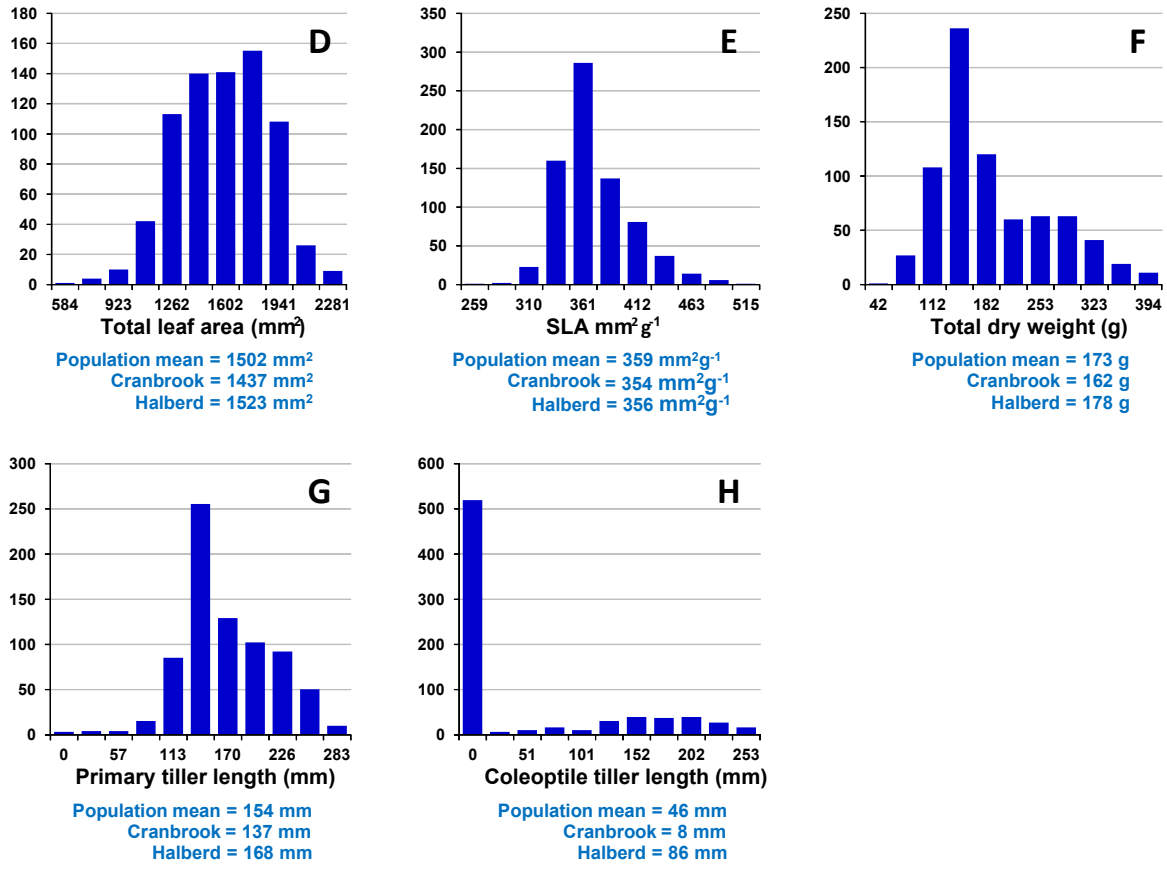

Figure 4. Frequency distributions of various early vigour traits for the Cranbrook/Halberd doubled-haploid bread wheat population. Traits shown are (**A**) Leaf 1 width; (**B**) Leaf 1 length; (**C**) Leaf 1 + 2 area; (**D**) Total leaf area; (**E**) Specific Leaf Area (SLA); (**F**) Total dry weight; (**G**) Primary tiller length; and (**H**) Coleoptile tiller length. Population and parental means are also given.

Variance components for the early vigour assessments in the Cranbrook/Halberd population are presented in Table 3. Seed weight accounted for significant variation in both leaf width and leaf area traits but was not significant for all other traits. All genetic variance components were significantly different from zero but several traits had large genotype × environment interaction variances, and this was reflected in the large range of reported narrow-sense heritabilities (line-mean basis). The highest line-mean heritabilities in this population were estimated for leaf 1 width and the leaf area traits (67% to 77%) while the smallest estimates were those for total dry weight, primary tiller length and coleoptile tiller length (33% to 47%).

As with the Sunco/Tasman population, the genetic correlations (Table 4) of embryo area with embryo width and length in the Cranbrook/Halberd population were large and positive (r_g = 0.88 ** and 0.89 **, respectively) while the correlation for length and width was moderate in size. Embryo trait correlations with early vigour in this population were commonly positive with the exception of leaf one length. The strongest association with embryo size was for leaf one and two combined area (r_g = 0.44 ** to 0.48 **). Another notable relationship was observed for coleoptile tiller length and both embryo length (r_g = 0.47 **) and embryo area (r_g = 0.43 **). The genetic correlation for leaf 1 width and total leaf area was large and significant (r_g = 0.83 **) (data not shown).

Table 3. Variance components (σ^2 ± standard errors) and narrow-sense heritability (on a line-mean and single-plant basis; ±standard error) for multi-environment assessment of early vigour parameters for the Cranbrook/Halberd doubled-haploid bread wheat population. The statistical significance of seed weight as a covariate for each trait analysis is also given.

Parameter	Embryo Width [A]	Embryo Length [A]	Embryo Area [A]	Leaf 1 Width [A]	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area [A]	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
σ^2_G	0.47 ± 0.07 **	1.43 ± 0.25 **	4.01 ± 0.66 **	4.43 ± 0.91 **	14.1 ± 3.6 **	4710 ± 894 **	230.7 ± 38.6 **	197 ± 97 *	252 ± 119 **	140 ± 47 **	844 ± 270 **
$\sigma^2_{G \times E}$	0.01 ± 0.01 ns	0.01 ± 0.02 **	0.12 ± 0.02 **	0.60 ± 0.30 ns	8.81 ± 3.4 **	1601 ± 602 **	72.8 ± 21.1 **	73 ± 12 **	822 ± 141 **	268 ± 48 **	813 ± 303 **
$\sigma^2_{residual}$	0.38 ± 0.05 **	0.93 ± 0.14 **	2.05 ± 0.30 **	11.1 ± 0.80 **	35.4 ± 3.1 **	6414 ± 554 **	187.8 ± 16.3 **	569 ± 90 **	610 ± 57 **	230 ± 22 **	3288 ± 251 **
$h^2_{line\text{-}mean}$	0.87 ± 0.11 **	0.90 ± 0.06 **	0.90 ± 0.06 **	0.67 ± 0.09 **	0.58 ± 0.09 **	0.72 ± 0.07 **	0.77 ± 0.07 **	0.61 ± 0.07 **	0.33 ± 0.07 *	0.45 ± 0.06 **	0.47 ± 0.07 **
$h^2_{single\text{-}plant}$	0.55 ± 0.04 **	0.61 ± 0.05 **	0.67 ± 0.06 **	0.27 ± 0.05 **	0.24 ± 0.05 **	0.37 ± 0.04 **	0.47 ± 0.06 **	0.23 ± 0.06 *	0.15 ± 0.04 *	0.22 ± 0.04 **	0.17 ± 0.03 **
Seed weight	*	**	**	*	*	*	*	ns	ns	ns	ns

*, ** Indicates statistical significance at $p = 0.05$ and 0.01, respectively; ns: non-significant at $p = 0.05$; [A] variance component × 100.

Table 4. Genetic correlations (r_g) for the embryo characteristics and early vigour parameters of the Cranbrook/Halberd doubled-haploid bread wheat population.

r_g	Embryo Width	Embryo Length	Embryo Area	Leaf 1 Width	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
Embryo width	-	0.51 **	0.88 **	0.36 **	−0.12 ns	0.48 **	0.29 *	0.19 *	0.29 **	0.27 *	0.28 *
Embryo length	-	-	0.89 **	0.31 **	−0.11 ns	0.44 **	0.27 *	0.21 *	0.25 **	0.24 *	0.47 **
Embryo area	-	-	-	0.35 **	−0.08 ns	0.45 **	0.22 *	0.21 *	0.21 *	0.18 *	0.43 **

*, ** Indicates statistical significance at $p = 0.05$ and 0.01, respectively; ns: non-significant at $p = 0.05$.

2.1.3. Vigour18/Chuan-Mai 18 Population

Large and statistically significant variation was observed for embryo size in the Vigour18/Chuan-Mai 18 DH population (Figure 5). This population produced the largest observed range in embryo widths (*ca.* 1.00 mm). The population range for embryo length (1.20 mm) was similar to that observed for the Cranbrook/Halberd population (1.21 mm) while embryo area (2.10 mm^2) was similar to the Cranbrook/Halberd (2.11 mm^2) but greater than for Sunco/Tasman (1.91 mm^2) populations. The population mean for embryo width (1.39 mm) was not statistically different to the mid-parent value (1.40 mm) whereas embryo length (2.21 mm) and embryo area (2.21 mm^2) population means were larger but not statistically different to their mid-parent means (2.17 mm and 2.18 mm^2, respectively).

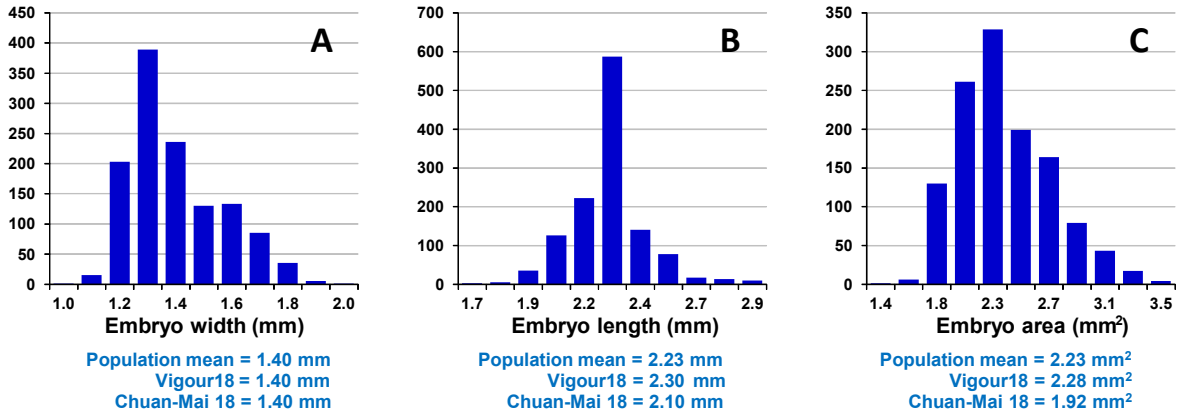

Figure 5. Frequency distributions of embryo characteristics for the Vigour18/Chuan-Mai 18 doubled-haploid bread wheat population. Traits shown are (**A**) Embryo width; (**B**) Embryo length; and (**C**) Embryo area. Population and parental means are also given.

Frequency distributions for genotype means for embryo traits are given in Figure 5. As for the two previous populations, there appears to be considerable transgressive segregation in this population for embryo width, length and area, and distributions were Gaussian in nature. Early vigour distributions for Vigour18/Chuan-Mai 18 progeny are given in Figure 6. Progeny means were normally distributed for most traits with the exception of primary and coleoptile tiller length. This contrasts significantly with the findings of both the Sunco/Tasman and Cranbrook/Halberd populations in that although each of these populations had many lines which did not produce a coleoptile tiller both populations contained many lines producing a primary tiller. The Vigour18/Chuan-Mai 18 population contained many lines which did not produce either a primary or coleoptile tiller. To determine if the same plants not producing a primary tiller were also those plants not producing a coleoptile tiller, the tiller length data was binarised (converted to "1" or "0") where "0" tiller length was scored a "0", and any tiller length greater than zero was scored "1". Pearson's correlation coefficient was estimated and the resulting correlation was 0.15 ($p > 0.05$). This would suggest there was no relationship between a plant's ability to produce a primary or coleoptile tiller.

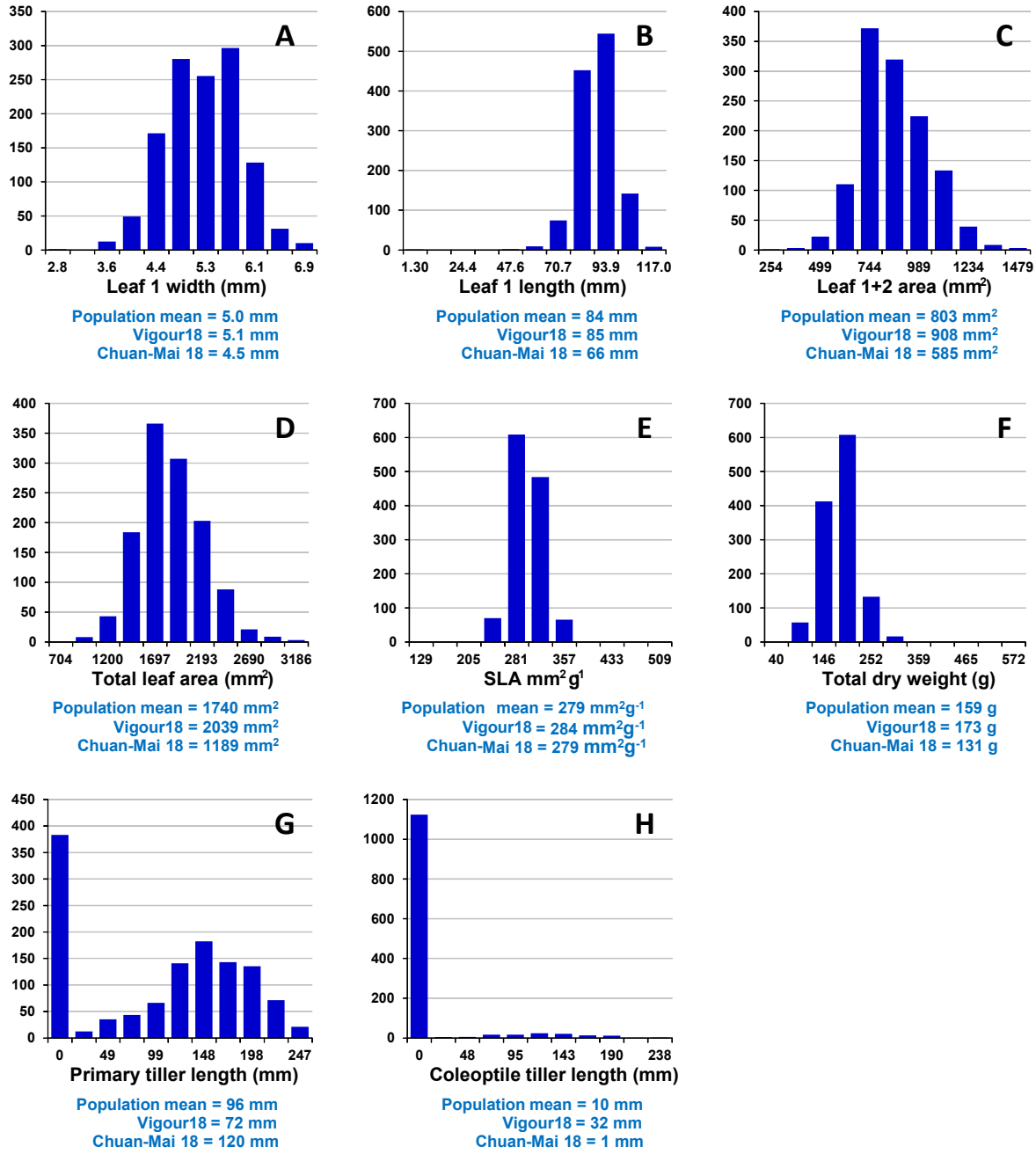

Figure 6. Frequency distributions of various early vigour traits for the Vigour18/Chuan-Mai 18 doubled-haploid bread wheat population. Traits shown are (**A**) Leaf 1 width; (**B**) Leaf 1 length; (**C**) Leaf 1+2 area; (**D**) Total leaf area; (**E**) Specific Leaf Area (SLA); (**F**) Total dry weight; (**G**) Primary tiller length; and (**H**) Coleoptile tiller length. Population and parental means are also given.

In the Vigour18/Chuan-Mai 18 population most trait means approximated the mid-parent mean. An exception to this was leaf 1 width which was closer in mean to that of the high leaf width parent (Vigour18), and specific leaf area where the population mean was similar to that of the low specific leaf area parent (Chuan-Mai 18). Considerable transgressive segregation was evident for all discussed traits measured on this population.

The genotypic variances for embryo traits in the Vigour18/Chuan-Mai 18 population were large and statistically different from zero (Table 5). The genotype × environment interaction variances were also significant but relatively small in size. Narrow-sense heritabilities (line-mean) for embryo length (83%) and embryo area (78%) were high and the estimate for embryo width somewhat smaller (68%). Seed weight was a significant covariate for embryo length and area but not for embryo width.

In the early vigour analysis of the Vigour18/Chuan-Mai 18 population (Table 5), seed weight was found only to be significant for the traits of leaf 1 width and coleoptile tiller length. Genotypic variances were significant for all traits with the exception of coleoptile tiller length. Leaf 1 width and leaf area traits had large genotypic variances while other traits (e.g., total dry weight and coleoptile tiller length) had comparatively large genotype × environment interactions and/or residual variances contributing to smaller narrow-sense heritabilities (line-mean). Heritabilities were highest for leaf width and areas (76% to 85%), and smallest for total dry weight (40%) and coleoptile tiller length (17%).

Genetic correlations for embryo dimension and vigour traits are listed for the Vigour18/Chuan-Mai 18 population in Table 6. As for the previously described populations, the genetic correlation of embryo width and length with embryo area was large and positive. When compared with Sunco/Tasman (r_g = 0.56 **) and Cranbrook/Halberd (r_g = 0.51 **), the relationship of embryo width with embryo length was smaller in this population (r_g = 0.33 **). Leaf area and leaf width was well correlated with embryo size although not as strongly as for total dry weight and coleoptile tiller length. This population also produced small albeit significant negative correlations for specific leaf area and embryo width and area, and primary tiller length and embryo width (data not shown).

Table 5. Variance components (σ^2 ± standard errors) and narrow-sense heritability (on a line-mean and single-plant basis; ±standard error) for multi-environment assessment of early vigour parameters for the Vigour 18/Chuan-mai 18 doubled-haploid bread wheat population. The statistical significance of seed weight as a covariate for each trait analysis is also given.

Parameter	Embryo Width [A]	Embryo Length [A]	Embryo Area [A]	Leaf 1 Width [A]	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area [A]	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
σ^2_G	0.21 ± 0.04 **	0.91 ± 0.13 **	1.78 ± 0.27 **	7.21 ± 1.13 **	29.5 ± 4.98 **	5459 ± 788 **	436.1 ± 59.4 **	67.4 ± 25.8 **	223 ± 121 *	1058 ± 290 **	37 ± 41 ns
$\sigma^2_{G \times E}$	0.06 ± 0.02 **	0.09 ± 0.03 **	0.38 ± 0.11 **	0.59 ± 0.37 *	2.9 ± 1.7 *	324 ± 161 *	15.0 ± 8.0 *	6.6 ± 12.5 ns	393 ± 155 **	644 ± 277 *	152 ± 50 **
$\sigma^2_{residual}$	0.04 ± 0.02 **	0.81 ± 0.04 **	1.82 ± 0.10 **	11.85 ± 0.66 **	79.1 ± 4.2 **	7211 ± 394 **	411.5 ± 22.7 **	651 ± 36 **	1916 ± 114 **	3785 ± 224 **	632 ± 37 **
$h^2_{line\text{-}mean}$	0.68 ± 0.09 **	0.83 ± 0.10 **	0.78 ± 0.10 **	0.76 ± 0.09 **	0.56 ± 0.07 **	0.80 ± 0.09 **	0.85 ± 0.10 **	0.37 ± 0.05 **	0.40 ± 0.05 **	0.53 ± 0.08 **	0.17 ± 0.06 *
$h^2_{single\text{-}plant}$	0.31 ± 0.05 **	0.50 ± 0.07 **	0.45 ± 0.07 **	0.37 ± 0.05 **	0.22 ± 0.05 **	0.42 ± 0.05 **	0.51 ± 0.06 **	0.09 ± 0.05 ns	0.14 ± 0.05 *	0.19 ± 0.05 *	0.05 ± 0.05 ns
Seed weight	ns	**	**	**	ns	ns	ns	ns	ns	ns	**

*, ** Indicates statistical significance at $p = 0.05$ and 0.01, respectively; ns: non-significant at $p = 0.05$; [A] variance component × 100.

Table 6. Genetic correlations (r_g) for the embryo characteristics and early vigour parameters of the Vigour18/Chuan-Mai 18 doubled-haploid bread wheat population.

r_g	Embryo Width	Embryo Length	Embryo Area	Leaf 1 Width	Leaf 1 Length	Leaf 1 + 2 Area	Total Leaf Area	Specific Leaf Area	Total Dry Weight	Primary Tiller Length	Coleoptile Tiller Length
Embryo width	-	0.33 **	0.78 **	0.40 **	0.26 *	0.34 **	0.32 *	−0.27 *	0.42 **	−0.20 *	0.25 *
Embryo length	-	-	0.85 **	0.32 *	0.26 *	0.28 *	0.20 *	−0.07 ns	0.43 **	0.23 *	0.47 **
Embryo area	-	-	-	0.41 **	0.29 *	0.37 **	0.30 *	−0.21 *	0.47 **	0.10 ns	0.43 **

*, ** Indicates statistical significance at $p = 0.05$ and 0.01, respectively; ns: non-significant at $p = 0.05$.

2.2. QTL Analyses

The chromosomal locations identified from the QTL analysis of embryo size and early vigour traits are shown for the Sunco/Tasman and Cranbrook/Halberd populations in Figures 7 and 8, respectively. Allelic effects and chromosomal location are given in Tables 7–9. It is important to note that although several publications have reported QTL for traits related to early vigour (e.g., [29–33]), to date there have been no QTL reported for embryo size in wheat. The QTL shown in green indicate the first parent (e.g., Sunco or Cranbrook) contributes a positive allele (e.g., increased embryo width) while a red QTL indicates the first parent contributes a negative allele (e.g., reduced embryo width) at the designated QTL.

Numerous QTL were identified for embryo size and early vigour in the Sunco/Tasman population (Figure 7). For example, Sunco contributes positive alleles for embryo size QTL on chromosomes 1B, 4D, 5A, 5D, 6B and 7D. Positive genetic effects for embryo area were located on chromosomes 1B, 5A, 5D and 7D (Table 9). Embryo size QTL with negative Sunco genetic effects (*i.e.*, positive genetic effects from Tasman) were identified on chromosomes 1A, 2A, 2B, 3B, 4A, 4B and 7B, and embryo area on chromosomes 2B and 4A. Key alleles for early vigour (total leaf area) with positive effect contributed by Sunco were located on chromosomes 1B, 3B, 4D, 5A and 7D. Alleles of significant positive effect contributed by the Tasman parent were identified on chromosomes 1A, 2B, 4B, 5B and 6B. A number of the embryo area QTL co-located with component measures of embryo width and/or length (Figure 7). These were most obvious on chromosome 1B, 2B and 7D.

In the Cranbrook/Halberd population, the embryo size QTL of positive genetic effect from parent Cranbrook were identified on chromosomes 1B, 2B, 2D, 4D, 6D, 7A and 7D (Figure 8), and embryo area on chromosomes 1B, 4D, 6D, 7A and 7D (Table 9). The QTL of negative genetic effect (positive effects from Halberd) were located on chromosomes 1A, 3B, 4B, 5B and 7D, and embryo area on 3B, 4B and 5B (Table 9). Cranbrook alleles of positive genetic effect for early vigour were identified on chromosomes 1B, 2D, 4D, 5D, 6B, 6D and 7A (Table 7). Important positive alleles contributed by Halberd in this population were identified on chromosomes 2B, 4A, 4B, 5A, 5B and 6A. As for the Sunco/Tasman population above, embryo width and length QTL co-located with genomic regions for embryo area on chromosomes 1B, 4B, 5B, 7A and 7D (Figure 8).

In many cases, positive QTL for total leaf area were co-located with increased embryo and leaf width (*cf.* Tables 7–9). This was evident for chromosome 1B and 7D where the Sunco parent contributed alleles for increased leaf area and both larger embryo and leaf one width. Equally, for Cranbrook on chromosomes 4B and 5B, and Halberd on chromosomes 4D and 7A. The Tasman allele co-locating leaf area, embryo size and leaf one width on chromosome 2B in the Sunco/Tasman population represents a *T. timopheevi* translocation inherited from Sunco.

It is noteworthy that common chromosomal regions were apparent across populations for total leaf area (chromosome 1B, 2B, 4B and 6B), leaf one width (3A, 4B, 5B and 6B) and embryo dimensions (1B, 2B, 4B and 7D) (Figures 7 and 8).

Table 7. Estimated additive (*a*) genetic effect, percent phenotypic variance, and chromosomal location of QTL for total leaf area measured on random doubled-haploid progenies from the Sunco/Tasman and Cranbrook/Halberd bread wheat populations evaluated across multiple sowings in favourable conditions. Positive additive effects indicate that the first parent allele (e.g., Cranbrook or Sunco) increases the value of the trait.

Chromosome [A]	Nearest Marker	QTL Position (cM)	*a* Genetic Effect [B] (mm^2)	% Phenotypic Variance
		Sunco/Tasman		
1AS	*bcd808*	41.3	−17.3 ¶	4
1BL	*gwm11*	83.2	53.4 **	8
2BS	*wmc35a*	54.1	−161.1 **	18 [QE]
3BS	*gwm566*	57.3	27.2 *	4
4BS	*Rht-B1*	14.6	−11.6 ¶	3
4DS	*Rht-D1*	2.9	44.5 *	5 [QE]
5AS	*gwm443*	7.4	59.1 **	7 [QE]
5BS	*psr167*	2.8	−54.7 **	7
6BS	*gwm644*	11.7	−38.7 *	4
7DS	*gwm295*	21.1	64.8 **	9
		Cranbrook/Halberd		
1BL	*gwm18*	61.3	35.5 ¶	2
2BS	*wPt-8004*	29.9	−68.7 **	4
2DL	*cdo366*	104.1	157.7 **	9
4AS	*wg622*	46.1	−59.0 *	4
4BS	*gwm6*	79.2	−49.3 ¶	3
4DS	*almt11*	21.1	53.2 *	3
5AL	*mwg514*	121.7	−133.4 **	8 [QE]
5BS	*stm518tctg*	85.4	−43.2 ¶	2 [QE]
5DS	*wPt-5505*	100.4	109.5 **	7
6AS	*mwg573*	34.1	−134.8 **	8 [QE]
6BS	*bcd1426*	14.3	87.4 **	5 [QE]
6DS	*wg933*	56.4	97.1 **	6 [QE]
7AL	*rga35*	195.4	101.5 **	6

[A] Chromosomes in bold contain leaf area QTL which appear to collocate across both populations; [B] *a* additive effect estimated as one-half the difference in homozygotes carrying either parental allele; ¶, *, ** indicates marker effect is statistically different from zero at p = 0.10, 0.05 and 0.01; QE denotes significant QTL × environment interaction.

Table 8. Estimated additive (*a*) genetic effect, percent phenotypic variance, and chromosomal location of QTL for leaf one width measured on random doubled-haploid progenies from the Sunco/Tasman and Cranbrook/Halberd bread wheat populations evaluated across multiple sowings in favourable conditions. Positive additive effects indicate that the first parent allele (e.g., Cranbrook or Sunco) increases the value of the trait.

Chromosome [A]	Nearest Marker	QTL Position (cM)	*a* Genetic Effect [B] (mm)	% Phenotypic Variance
		Sunco/Tasman		
1AS	*glu-A1*	26.0	−0.09 **	4
1BL	*gwm11*	83.9	0.09 **	5
2AS	*gwm588*	6.9	−0.07 **	4 [QE]
2BS	*wmc35a*	54.0	−0.32 **	14
2DS	*wmc190*	64.7	−0.12 **	6
3AL	*P39-M50-152*	87.2	0.10	5
4AS	*wg232a*	42.3	0.07 *	3
4BS	*Rht-B1*	12.0	−0.06 *	3
4DS	*Rht-D1*	3.7	0.09 **	5
5AL	*wg232c*	90.1	−0.06 *	3
5BS	*P36-M40-1*	24.2	−0.05 *	3
6BS	*gwm626*	12.1	−0.05 *	3
7DS	*gwm295*	0.0	0.04 *	3
		Cranbrook/Halberd		
2DS	*gwm261*	14.2	0.09 *	5
3AL	*rga61.2*	113.7	−0.15 **	11 [QE]
3DL	*cfd70*	88.2	0.11 *	8 [QE]
4BS	*Rht-B1*	64.1	−0.07 ¶	4
4DL	*almt11*	20.7	0.11 *	5
5BL	*cdo400*	95.1	−0.12 **	9
6AS	*mwg573*	39.1	−0.18 **	14 [QE]
6BS	*cdo1091b*	37.1	0.12 *	9
7AL	*rga35*	197.3	0.17 **	13

[A] Chromosomes in bold contain leaf one width QTL which appear to collocate across both populations; [B] *a* additive effect estimated as one-half the difference in homozygotes carrying either parental allele; ¶,*,** indicates marker effect is statistically different from zero at p = 0.10, 0.05 and 0.01; QE denotes significant QTL × environment interaction.

Table 9. Estimated additive (*a*) genetic effect, percent phenotypic variance, and chromosomal location of QTL for embryo area measured on random doubled-haploid progenies from the Sunco/Tasman and Cranbrook/Halberd bread wheat populations evaluated across multiple sowings in favourable conditions. Positive additive effects indicate that the first parent allele (e.g., Cranbrook or Sunco) increases the value of the trait.

Chromosome [A]	Nearest Marker	QTL Position (cM)	*a* Genetic Effect [B] (mm^2)	% Phenotypic Variance
		Sunco/Tasman		
1BL	*gwm11*	93.2	0.53 **	8
2BS	*wmc35a*	54.1	−1.61 **	18
4AS	*gwm610*	17.3	−0.27 *	4
5AS	*gwm443*	7.4	0.59 **	7
5DS	*gwm190*	2.8	0.55 **	7
7DS	*gwm295*	4.7	0.39 *	4
		Cranbrook/Halberd		
1BL	*gwm18*	59.7	1.10 **	8
3BL	*ksug59*	96.1	−0.81 *	6
4BS	*cdo669b*	47.3	−0.92 **	7
4DS	*wmc48b*	6.7	0.51 *	4
5BL	*wmc289*	115.9	−0.90 **	7
6DS	*wmc416*	53.1	1.22 **	11
7AL	*psr121*	173.4	0.93 **	7
7DS	*wmc157*	193.4	0.41 ¶	4

[A] Chromosomes in bold contain embryo area QTL which may collocate across the two populations; [B] *a* additive effect estimated as one-half the difference in homozygotes carrying either parental allele; ¶,*,** indicates marker effect is statistically different from zero at $p = 0.10$, 0.05 and 0.01.

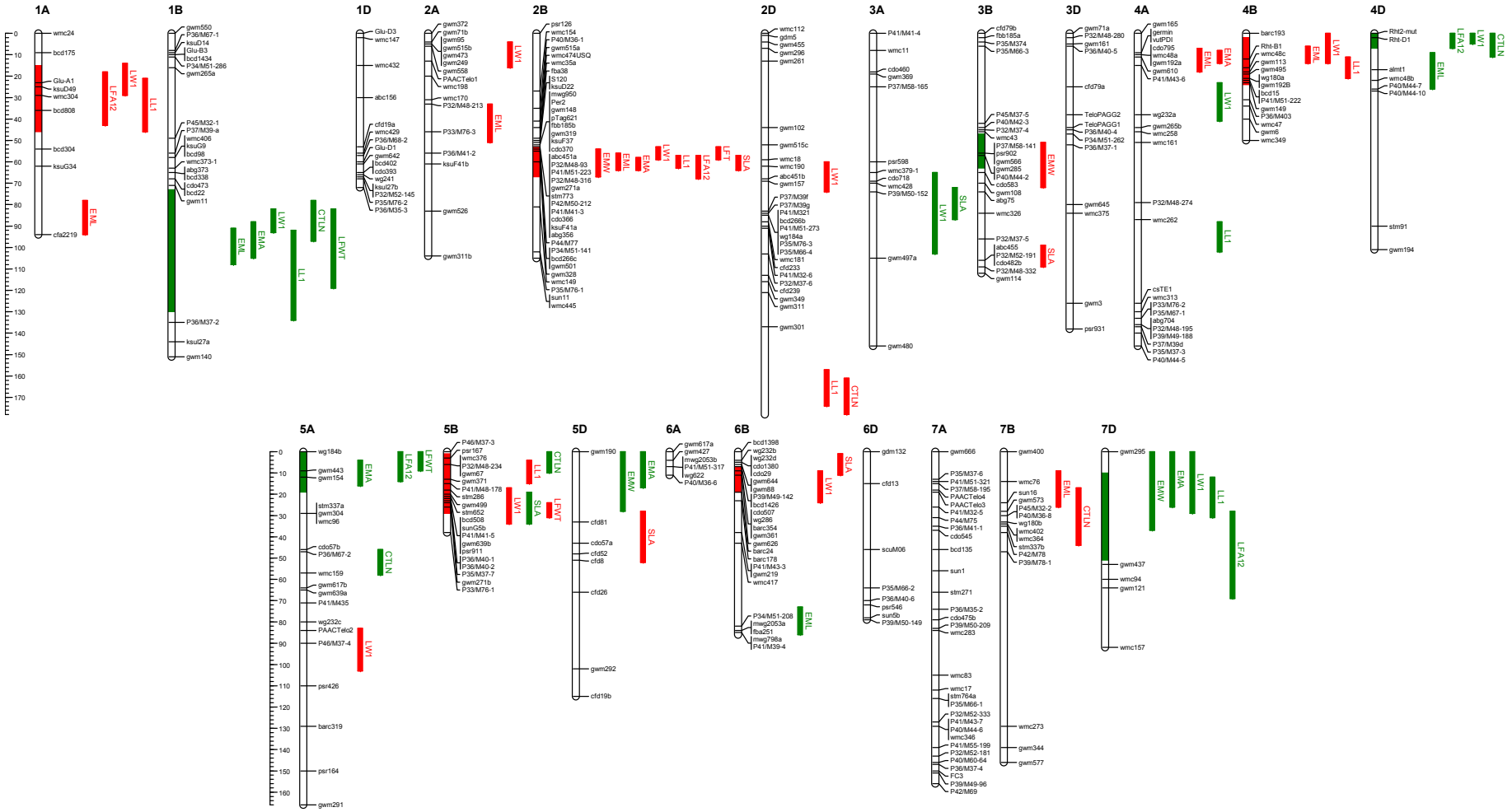

Figure 7. Chromosomal locations of QTL for embryo size and early vigour from the Sunco/Tasman bread wheat mapping population. QTL are indicated to the right of the linkage group, and the coloured bars indicate significant ($p = 0.01$) QTL. Red and green colouring indicates negative and positive genetic effects respectively from the Sunco parent. QTL located on the chromosomes represent total leaf area. Other traits illustrated are: EMA = embryo area; EML = embryo length; EMW = embryo width; SLA = specific leaf area; LW1 = Leaf one width; LL1 = Leaf one length; LFA12 = leaf 1 + 2 area; LFWT = total leaf weight; CTLN = coleoptile tiller length.

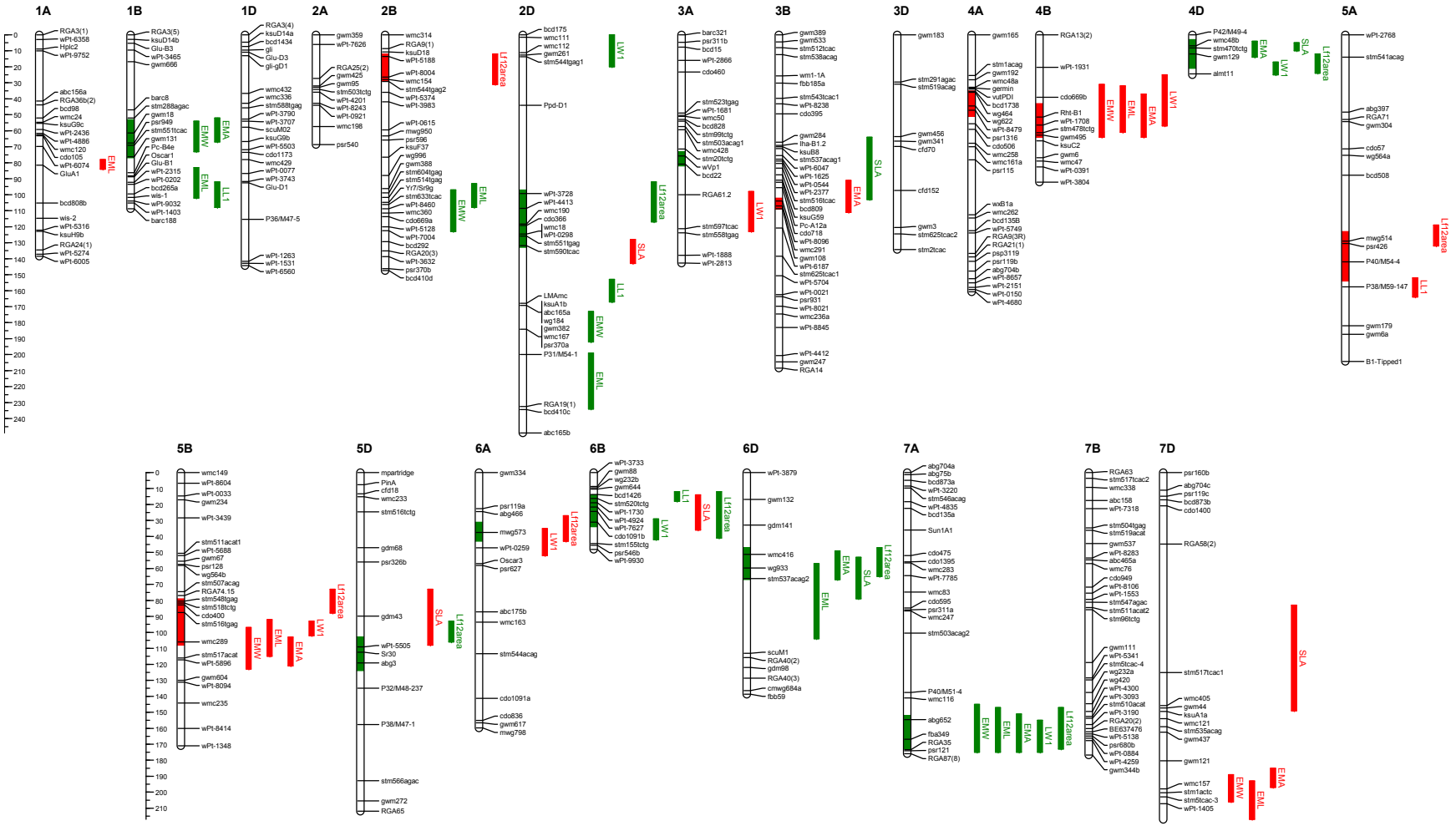

Figure 8. Chromosomal locations of QTL for embryo size and early vigour from the Cranbrook/Halberd bread wheat mapping population. QTL are indicated to the right of the linkage group, and the coloured bars indicate significant (p = 0.01) QTL. Red and green colouring indicates negative and positive genetic effects respectively from the Cranbrook parent. QTL located on the chromosomes represent total leaf area. Other traits illustrated are: EMA = embryo area; EML = embryo length; EMW = embryo width; SLA = specific leaf area; LW1 = Leaf one width; LL1 = Leaf one length; LF12Area = leaf 1 + 2 area; LFWT = total leaf weight; CTLN = coleoptile tiller length.

3. Discussion

Embryo size is well established to be correlated with seed size in wheat [6,16,19]. The significance of seed weight in modifying phenotypic expression of many traits analysed in these studies highlights the importance of standardising to a common seed size when selecting for either embryo size or early vigour. By careful harvesting and threshing of seed from common environments, taking great care in ensuring seed weight was restricted to within a very small range of 5mg or less, and then careful statistical analysis of well-designed experiments, we were able to detect small but repeatable genetic differences and allelic effects for embryo size and early vigour traits. It could be argued that the ranges in seed weight were not small enough and should be decreased further. However, standardising is slow and time-consuming in large populations necessitating the fitting of covariates to increase confidence in the robust estimation of genetic effects.

3.1. Embryo Size

A large range in embryo size traits was observed in all three populations with variation commonly exceeding that of the means of the parents. This transgressive segregation likely reflects the accumulation of favourable and unfavourable alleles from either parent to reduce or increase the genotypic value of individual lines. Further, the high heritabilities increase confidence that extremes in progeny values are real and likely to be repeatable. It is apparent from the QTL mapping that assessed traits were polygenic in nature with each parent containing both positive and negative alleles for each trait. Further, population sizes were reasonably large enough to identify unique combinations of alleles toward contrasting genotypes.

Narrow-sense heritabilities on a line-mean basis were commonly large for embryo size traits in all three genetic backgrounds. Genotype × environment interaction was not large although given the same seed source was used for each environment any interaction can largely be viewed as sampling variance. The high heritabilities are encouraging from a breeding perspective as genetic gain from selection for embryo size is likely to be high.

In all populations the relationship of embryo width to embryo length was positive but moderate in size unlike the stronger relationship of embryo area to width and length. There is a degree of auto-correlation in this result as embryo area was derived from components width and length. However, the strong relationship suggests that selection for embryo area could be used not only as a means of increasing embryo size but also as a means of indirectly increasing embryo width and length. The smaller relationship of embryo width to embryo length suggests that it is possible to select for these traits either independently, or in combination.

3.2. Early Vigour

Both phenotypic and genotypic variation were large for many of the early vigour traits assessed in the three populations. The most notable exceptions to this were coleoptile tiller length (in all three populations), and primary tiller length in the Vigour18/Chuan-Mai 18 population. The reduced genetic variance for these traits may reflect a genetic predisposition to the production of fewer tillers. However,

it must also be considered that the populations may not have had adequate time to develop tillers despite later sampling at the fourth-leaf growth stage.

As was reported for embryo traits, transgressive segregation was observed for early vigour traits in all populations. Indeed, traits associated with leaf widths and area typically had large and significant genetic variance components. Multi-environment assessment of early vigour in the three populations identified that genotype × environment interactions were large and statistically significant for a number of traits. The traits most commonly impacted by these interactions were total dry weight, primary tiller and coleoptile tiller lengths. Residual variances for early vigour traits were generally much higher than those for embryo traits, and although great care was taken with these experiments, may reflect noise generated by sampling techniques or inherent to the methodologies used.

Narrow-sense heritabilities on a line mean basis were generally larger for leaf width and leaf area traits in all populations, and are consistent with higher heritabilities reported elsewhere (e.g., [35]). The heritabilities were typically smaller for traits associated with tiller length. Specific leaf area was variable with almost a two-fold range in heritability between the Cranbrook/Halberd and Vigour 18/Chuan-Mai 18 populations. Heritabilities are themselves parameter estimates specific to a population of genotypes and environments. As such, heritabilities can vary and their value in prediction of genetic gain becomes a function of the relevance of the parents used in generating the population and the relevance of the environments sampled [36].

Seed size has been reported to influence early vigour and seedling characteristics in wheat [16,20,25] while few studies have examined the relationship of embryo size and early vigour. In comparisons between barley, oats, wheat and triticale, the size of the embryo accounted for *ca*. 90% of the early vigour differences among the species [6]. Similarly, studies contrasting few wheat genotypes highlighted a modest phenotypic relationship for embryo size and early growth in durum [25] and bread [19,22] wheats. Genetic correlations reported herein are based on variation and covariation estimated for related genotypes and across diverse wheat populations. Herein, genetic correlations for embryo traits with early vigour were largely positive with only few significant negative correlations identified (e.g., specific leaf area and embryo width and area, and primary tiller length and embryo width in the Vigour 18/Chuan-Mai 18 population). The vast majority of positive correlations were small or moderate in size with the largest identified for embryo size and both leaf one width and leaf area. In the Vigour 18/Chuan-Mai 18 population, strong correlations for embryo size with total dry weight and coleoptile tiller length were observed yet these differed markedly for the same traits assessed in the Sunco/Tasman and Cranbrook/Halberd populations. Genetic correlations reflect both the additive genetic variance and covariance for traits used in estimation. All three parameters are themselves contingent on the populations and environments sampled and so genetic correlations can vary in size and nature depending on the experiment and the genes segregating.

3.3. QTL Analyses

Multiple loci have been identified in the Sunco/Tasman and Cranbrook/Halberd wheat populations for embryo size and early vigour. Further, many of the loci identified for embryo traits were found to co-locate with loci identified for early vigour traits. In the Sunco/Tasman population, chromosomes 1B, 2B and 7D appear to be important in this respect while in the Cranbrook/Halberd population

chromosomes 1B, 4B, 4D, 5B, 6D, and 7A are of interest. The co-locating of QTL for embryo size and early vigour could be coincidental (*i.e.*, separate genes but closely mapped), or it is possible that some of the QTL may reflect the same gene affecting multiple traits through pleiotropic gene action. From this analysis, the QTL on chromosome 1B could be of further interest. Many of the genetic effects were small accounting for small portions of the phenotypic variance. This is not surprising and is consistent with many other reports of repeatable albeit small genetic effects in mapping populations (e.g., [37]). However, the use of mapping here to characterize a population in trait genetic architecture is of value in understanding genetic complexity (both for the trait in trait-trait associations), and potential in identifying new alleles of larger effect at key loci for use in breeding.

As embryo size QTL have not been previously reported, there was no opportunity for comparisons of the current work with findings elsewhere. That aside, QTL mapping in two separate populations revealed that the separate and somewhat independent measures embryo width and length contribute genetically to embryo area (and embryo mass - Chris Moore unpublished data). A number of researchers have previously reported QTL for early vigour in wheat. For example, several early vigour QTL were reported in the Cranbrook/Halberd population [29]. Chromosome 5BL contained a QTL for leaf 1 width, and chromosomes 4BL, 2DL and 5AL contained QTL for leaf one length. For the QTL analysis of the Cranbrook/Halberd population in this research, significant QTL for early vigour traits were identified on chromosome 5B, including leaf 1 width total leaf area, and embryo dimension traits. Chromosome 4B was also found to contain a leaf one width QTL in this research together with different embryo dimension traits. The same region was also associated with variation in leaf area and both embryo and leaf size in the Sunco/Tasman population. The *Rht-B1b* (*Rht1*) dwarfing allele is located in this region and is known to affect leaf area through changes in leaf size primarily leaf length [29].

This research also identified a leaf one length QTL on chromosome 2D. Coleman *et al.* (2001) [13] reported chromosome 2D having significant QTL for leaf one and two attributes in the Cranbrook/Halberd population. This coincides with the location of QTL for the same population in this research, as QTL for leaf one width, leaf one length and combined leaf area of leaves one and two were also identified on this chromosome. In the Sunco/Tasman population, a QTL for leaf one width from the Tasman parent was also identified on chromosome 2D. In field studies aimed at indirect assessment of early vigour via canopy measures of Normalized difference vegetation index (NDVI) and ground cover, up to 37 QTL of commonly small genetic effect were reported [33]. Many of the regions reported in their study (e.g., 1B, 2D, 4B and 5B) were located on the same chromosome arms reported in the Sunco/Tasman and Cranbrook/Halberd populations.

4. Experimental Section

Phenotypic and genetic analyses were conducted on three wheat (*Triticum aestivum* L.) doubled-haploid (DH) populations. Benefits of doubled-haploids (DH) for genetic studies are numerous. However, in this study, the homozygous individuals constituting the tested populations enabled assessment of additive (and potentially additive × additive) genetic variance in backgrounds free of dominance gene action [36]. Multiple environments were also employed to assess the impact of genotype × environment interactions, and the extent to which differential genotype response could affect narrow-sense heritability for the traits assessed.

The three DH populations sampled were: (1) Sunco/Tasman; (2) Cranbrook/Halberd; and (3) Vigour18/Chuan-Mai 18. The first two populations were designed and developed for the Australian Wheat Molecular Marker Program for the expressed purpose of marker development and trait (primarily grain quality) genetic dissection. Many markers have been publically developed and mapped in these populations for this purpose. Details of the development of the populations is as described previously [38].The third population (Vigour18/Chuan-Mai 18) was developed to better understand the relationship of early vigour and weed competitiveness. This population was not genotyped and so no QTL mapping was undertaken for this population. Embryo size and early vigour characteristics were assessed in all three populations.

4.1. Embryo Size and Early Vigour Assessment

Seed for all experiments were obtained from a common glasshouse sowing where parents and progeny were grown under favourable conditions, and then harvested and carefully hand-threshed to avoid any damage to seed. Owing to the relationship between seed and embryo size, and also the potential relationship for seed size and early vigour [39], all seed were standardised on the basis of seed weight prior to embryo assessment to a common range. The numbers of DH lines sampled for each population were 151 (Vigour 18/Chuan-mai 18), 155 (Cranbrook/Halberd) and 202 (Sunco/Tasman). Embryo sizes in this experiment were obtained using the microscope-based characterisation method described previously [39]. Maximal embryo width and maximal embryo length data was obtained (Plate 1), and an estimate for embryo area defined calculated as embryo width × embryo length × 0.72 after [39]. The same seed source for embryo sizing was used for early vigour assessments and care was taken to ensure seeds assessed for embryo size were linked directly to the same plant grown and assessed to produce the early vigour data.

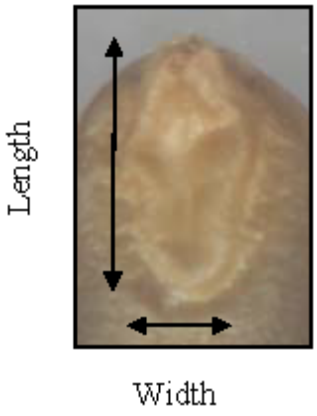

Plate 1. Embryo length and width.

The method for phenotyping early vigour was based on a modified version of the non-destructive system described in [40]. Seedling tray studies were conducted outdoors under well-watered conditions in Canberra with sowing dates in June of consecutive years (2000 and 2001) with a third environment for the Vigour18/Chuan-Mai 18 in June 2003. Owing to the numbers of lines in each population, each replicate of the DH lines, parents and controls were sown across two trays within a replicate. Each tray

consisted of a soil comprising a 50:50 proportion of compost to vermiculite, and was a paper-lined wooden box approximately 600 × 300 × 110 mm containing 105 plants.

Seed of each genotype used in the seedling trays was of known weight. Seed of each genotype used in the seedling trays was of known embryo dimensions. A row-column, alpha-lattice experimental design was used to allocate entries within each tray. For all populations, two replicates of each doubled-haploid line were grown in environment one and three replicates per line were used in environment two and three (Vigour18/Chuan-Mai 18 population only). To balance the sowing design within each environment and populations split across seedling trays, multiple repeated parent and other controls were used. Plants were grown under favourable conditions and harvested at the 3.5 leaf stage. Detailed measures were made at harvest of numbers of leaves, leaf width and length of all leaves, mainstem and coleoptile tiller numbers and size after [40]. Leaf area was calculated as the product of maximal leaf width and length, and a shape correction factor of 0.80 [40]. Despite many more detailed measurements being taken, for the purposes of clarity only eight traits representative of early vigour assessment have been included for reporting (e.g., Table 1 and Figures 1 and 2).

4.2. Statistical and QTL Mapping Analyses

Variance components and their standard errors were estimated using the method of restricted maximum likelihood and mixed linear models in the SAS procedure MIXED [41]. Narrow-sense heritabilities (h^2) and their standard errors were calculated on a single-seed or plant, and line-mean basis after [42]. Full models included random effects for environment, genotype and their interaction. Replicates within environments were considered random effects. Data for key embryo and early vigour traits were analysed for QTL mapping in the populations Sunco/Tasman and Cranbrook/Halberd. The genotyping was undertaken and maps carefully curated to develop robust maps as described in [43] with each map containing between 400 and 800 SSR and other markers. Adjusted means for embryo size and early vigour characteristics were analysed in MultiQTL® using multiple interval mapping techniques. Probability into and out of the model was 0.05 and window size set at 10 cM. Significant thresholds for QTL detection were calculated for each dataset using 1000 permutations [44]. Locations of genetic effects for QTL were identified from maps using MapChart 2.1 [45]. Unless indicated all statistical significance is reported for an alpha of 0.05.

5. Conclusions

These studies have highlighted the power in coupling quantitative genetic and QTL mapping in early vigour dissection and trait identification. High narrow-sense heritabilities for all embryo traits in each population confirms both the robustness of the methodologies used, and the confidence with which selection could be made for embryo size given the consistency in heritability across the different populations. That aside, multi-environment assessment of early vigour highlighted that higher heritabilities for some traits (e.g., leaf width) indicate greater opportunity for genetic gain than other less heritable traits (e.g., specific leaf area and coleoptile tiller length). Genetic correlations for embryo size and early vigour were generally positive but low to moderate in size and not always consistent across populations. Many of the QTL and genomic regions for early vigour were repeatable across environments and the different genetic backgrounds, and similar to those reported in other populations

in other studies. These studies highlight the genetic complexity of early vigour and the value of selection for greater embryo and leaf size in genetic gain for early leaf area.

Author Contributions

Chris Moore conducted experiments, collated and interpreted all data and wrote manuscript. Greg Rebetzke sourced populations, provided guidance and assisted in statistical and genetic analysis.

Conflicts of Interest

The authors declare no conflict of interest.

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