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ORIGINAL PAPER

A QTL on chromosome 6A in bread wheat (*Triticum aestivum*) is associated with longer coleoptiles, greater seedling vigour and final plant height

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Abstract Wheat crops with greater early vigour shade the soil surface more rapidly and reduce water loss. Evaporative losses affect water-use efficiency particularly in drier regions where most of the rainfall occurs early in the growing season before canopy closure. Greater seedling leaf area and longer coleoptiles are major determinants of increased vigour and better crop establishment. A previously developed high vigour breeding line 'Vigour 18' was used to establish a large recombinant inbred family and framework map to identify a QTL on chromosome 6A that accounted for up to 8% of the variation for coleoptile length, 14% of seedling leaf width and was associated with increased plant height. The SSR marker NW3106, nearest to the 6A QTL, was also associated with greater leaf width in a breeding population that was also derived from a cross involving the high vigour donor line 'Vigour18'. The association between the NW3106 marker and coleoptile length was validated in a second breeding population which was developed using an unrelated long coleoptile donor line. The 'Vigour18' allele of the QTL on chromosome 6A promoted coleoptile length and leaf width during early plant growth but was also associated with increased plant height at maturity. Markers linked to the QTL are being used to increase the frequency of increased vigour and long coleoptile alleles in early generations of breeding populations.

Keywords Wheat · Vigour · Coleoptile · QTL analysis

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Introduction

Good crop establishment requires shoots to emerge from the soil and develop leaf area rapidly. Wheat cultivars with more vigorous early growth shade the soil surface faster and reduce evaporative losses, a particularly important characteristic for drier regions with a Mediterranean climate where most of the rainfall occurs early in the growing season before canopy closure (Richards et al. 2002). Rapid early growth also improves weed competitiveness of the crop by reducing weed growth. The size of the embryo had a major effect on the development of early leaf area (Lopez-Castaneda et al. 1996), but seed weight was also consistently associated with larger embryos and hence greater vigour (Richards and Lukacs 2002). The development of greater leaf area for the same weight of leaf (high specific leaf area or SLA) was also identified as an important component of vigour (Lopez-Castaneda et al. 1995). It was proposed that measuring the width of seedling leaves would integrate variation for embryo size and SLA, and therefore provide an efficient selection tool for early vigour (Lopez-Castaneda et al. 1996). The width of seedling leaves was highly correlated with leaf area and was the most heritable component of overall leaf dimensions (Rebetzke and Richards 1999). Measuring leaf width has therefore been used to select wheat lines with increased vigour.

Wheats with long coleoptiles emerge with higher frequency than those with short coleoptiles especially when sown deep or where stubble has been retained (Allan et al. 1962; Rebetzke et al. 2005). Previous studies established strong negative correlations between the presence of dwarfing genes *Rht-B1b* or *Rht-D1b* and coleoptile length and seedling vigour (Whan 1976; Allan 1980; Richards 1992; Rebetzke et al. 2001). A previously developed tall line 'Vigour18' that lacked *Rht-B1b* or *Rht-D1b* produced longer coleoptiles and a larger leaf area than the best current Australian wheat cultivars (Richards and Lukacs 2002). The objective here was to identify genomic regions that contribute to increased vigour in the absence of these major dwarfing genes and develop markers to assist in trait selection.

Materials and methods

Plant material

A family of 460 recombinant inbred lines (RILs) was developed from a cross between a Chinese semi-dwarf wheat 'Chuan-Mai18' (CM18) carrying the GA-sensitive dwarfing gene *Rht8* and a tall breeding line 'Vigour18' (male parent) which has been bred and selected as a source of high seedling vigour (Richards and Lukacs 2002). A F5 family was developed by single seed descent for subsequent genotyping (see below).

Leaf width, coleoptile length and plant height

Using single seed descent, F6 (2004) and F7 progeny (2005) were developed from the above family and measured for seedling growth. To reduce the effect of seed weight on early vigour, seed was weighed between 40 and 45 mg for leaf width and coleoptile length measurements. For leaf width measurements seed was sown in 100 mm deep wooden trays (600 mm × 300 mm) containing 50:50 fertile compost/vermiculite soil mix and grown outside during the winter months (May–June) in Canberra, Australia (daily mean temperatures ranging from minimum 2°C to maximum 16°C). Seedlings were grown for 5–6 weeks until the 4.0–4.5 leaf stage before the width of leaf 3 was recorded at the widest point along the leaf blade. Two replicates of 323 lines together with parental lines were analysed in 2004 and three replicates of 368 lines in 2005 (which included most of lines grown in 2004). For coleoptile length measurements, seed was sown in 100 mm deep wooden trays (50:50 fertile compost/vermiculite soil mix) and grown at 15°C for 14 days in the dark before coleoptile lengths were measured. Three replicates of 363 lines together with parents were analysed in 2004 and 4 replicates of 439 lines in 2005 including lines measured in 2004. Mean values within years were used for subsequent QTL analysis. Subsets of 342 and 105 lines were grown as unreplicated field plots at Griffith and Gundibindyal in south-western NSW (Australia) respectively, to record final plant height. Field trials were sown in early June 2005 and received average rainfall during the growing season. Maturity differences were recorded at ear emergence using the Zadoks scale (data not shown) and leaf width measurements were made at Griffith on three to four plants per plot at the 4 to 5 leaf stage (Zadoks et al. 1974). Field planted seed was not standardised for seed weight.

Framework map construction and QTL analysis

DNA was extracted from freeze dried leaves as described by Ellis et al. (2005). DNA amplification was carried out in a GeneAmp 9700 thermal cycler (Applied Biosystems) using denaturation at 94°C for 2 min followed by 40 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C. One additional cycle was performed at 72°C for 5 min for final elongation of the PCR product. Each PCR reaction of 10 µl contained 50–100 ng of template DNA, PCR Mix 1×, 1.7 mM MgCl2, 200 nM of each primer, 250 µM each dNTPs and 0.3 U Taq polymerase (Invitrogen). PCR products were separated on 3% Resophor Agarose Eurobio, 1× TBE with a cooling system, 1–2 h at 360 V and gels were colored after electrophoresis with Ethidium Bromide for 30 min for fragment vizualisation. Some primers were labelled with fluorescent FAM, HEX or NED for PCR separation on an ABI 3700 DNA analyser 96 capillary arrays and fragments analysed with Genescan and Genotyper software (Applied Biosystems). Simple sequence repeat (SSR) wheat markers for this study were provided by Syngenta Seeds SAS and comprised a mixture of proprietary markers, publicly available as well as SSR markers licensed from various sources. Primer sequences of publicly available SSR markers are listed on the Graingene database (http://www.wheat.pw.usda.gov/GG2/index.shtml). Requests for other primer sequences should be made to F. Azanza, Syngenta Seeds, Toulouse. DNA from parental lines CM18 and Vigour18 was screened with 943 SSR markers of known map location predicted to cover most of the wheat genome. Polymorphism was found for 263 markers, of which 244 markers were positioned using 460 RILs into 31 linkage groups that were located on 21 wheat chromosomes (Fig. 1). The predicted marker order was confirmed and genetic distances were estimated using MapMakerV3.0 software (Lander et al. 1987). Linear regression was used to identify putative QTLs followed by Composite Interval Mapping function in QTL Cartographer V2.5 (http://www.statgen.ncsu.edu/qtlcart/WQTLCart.htm). Significance thresholds were determined by permutations

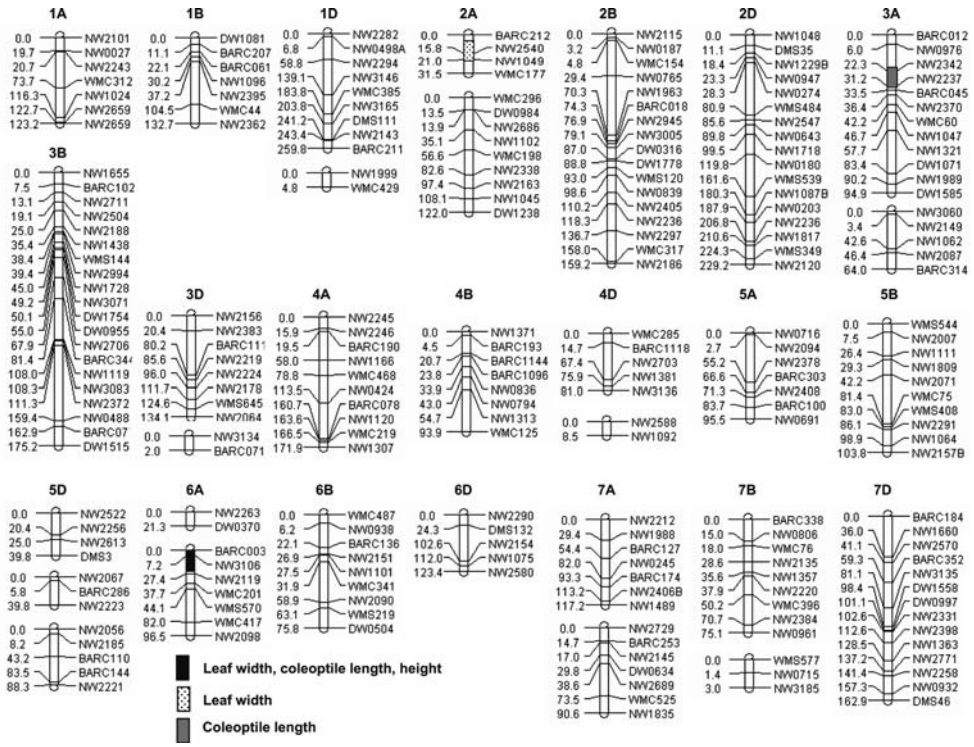

Fig. 1 Genetic linkage map of SSR markers and 460 recombinant inbred lines derived from a cross between 'Chuan Mai18' and 'Vigour18'. Genetic distances (cM) indicated on the *left hand side*. QTLs for leaf width, coleoptile length and height are indicated on chromosome *2A*, *3A* and *6A*

(1,000×) for all QTLs resulting in Likelihood ratio values ranging from 14 to 15 ($P = 0.05$). QTLs were only reported for coleoptile length and leaf width when significant across years.

QTL validation

A breeding population of 104 F3 lines was derived from a Vigour18/2*Janz8-28 backcross population. Although closely related to the Australian cultivar Janz, the semi-dwarf Janz 8-28 line carried *Rht8* instead of *Rht-B1b*. F3 seed weighing between 38 and 48 mg were sown in wooden trays as previously described to record leaf 3 widths. DNA was extracted from freeze-dried leaf tissue according to Ellis et al. (2005) and PCR products resolved using standard agarose gel electrophoresis.

A second breeding population of 76 F5:6 lines was derived from a backcross between a long coleoptile donor line HM10S carrying *Rht8* and the short coleoptile recurrent parent Sunlin (*Rht-D1b*). The 'HM10S' line was selected from a cross between the tall, long coleoptile Australian cultivar Halberd and semi-dwarf cultivar Mara carrying *Rht8*. The *Rht-D1b* dwarfing gene was eliminated from F6 lines in previous generations using the previously developed marker for *Rht-D1b* (Ellis et al. 2002). Four seed of each F6 line were screened for coleoptile length (see above) while marker analysis was performed on DNA extracted from an additional F6 seed according to the seed extraction protocol outlined in Ellis et al. (2005). The mean values for coleoptile length were used for confirmation of marker/trait association. Co-ancestry estimates between 'Mara', 'Halberd' and 'Vigour18' were obtained from the IWIS database (http://www.mendel.lafs.up.edu.au).

Results

The width of leaf 3 and coleoptile length comprise two important components of vigour. Across different environments the vigorous parental line 'Vigour18' produced on average a 40% longer coleoptile and 34% wider leaf 3 than the lower vigour parent 'CM18' (Table 1). A population of 460 RILs derived from a cross between

Table 1 Comparison of leaf width, coleoptile length and plant height between parental lines 'Chuan-Mai18' and a high vigour breeding line 'Vigour18'

	Leaf width (mm)	Coleoptile length (mm)	Height (cm)	Zadoks
Chuan-Mai18				
Mean	6.5 ± 0.06	106 ± 6	84 ± 1.7	
Range	5.8–7.6	90–125	75–95	49–52
Vigour18				
Mean	8.7 ± 0.1	149 ± 7	115 ± 1.5	
Range	7.3–9.7	125–170	110–120	57–63

Data based on experiments conducted in 2005, standard errors are included for mean values

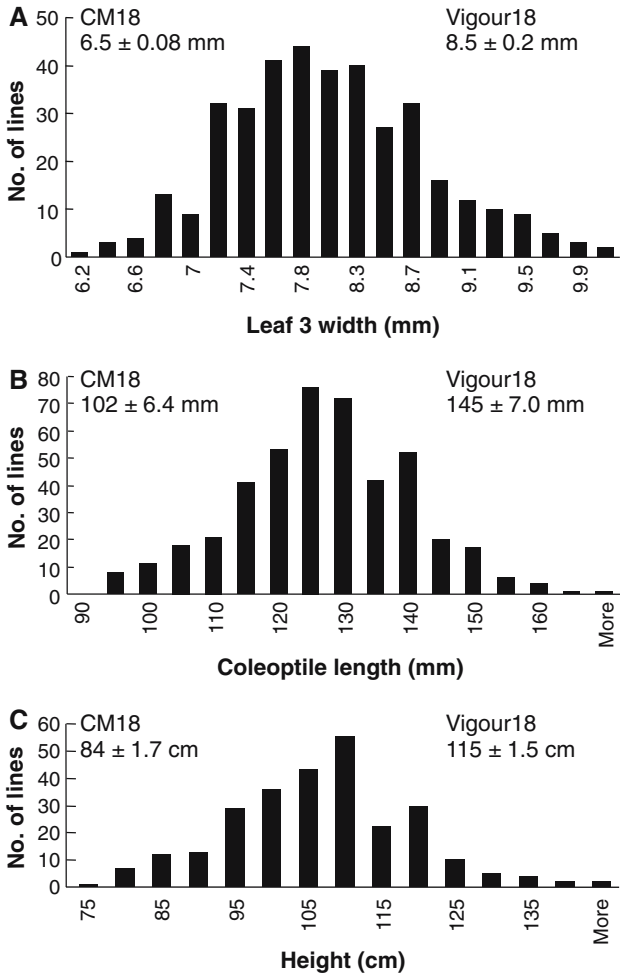

Fig. 2 Frequency distributions for leaf width 3 (**a**), coleoptile length (**b**) and final height (**c**) of F7 progeny derived from the cross between 'Chuan Mai18' and 'Vigour18'. The parental mean values with standard errors were included

'Vigour18' and 'CM18' segregated for leaf width and coleoptile length and subsets of RILs were subsequently phenotyped for both traits. Mean values for both traits were continuous and normally distributed showing evidence for transgressive segregation (Fig. 2). Leaf width values were significantly correlated ($r = 0.6$, $P < 0.01$) across years (Table 2). Coleoptile lengths showed lower correlation ($r = 0.37$, $P < 0.01$) suggesting stronger influence of environmental effects on the expression of the trait. No significant correlation was found between leaf width and coleoptile length.

Parental lines and subsets of RILs were also grown at two field sites to determine plant height and flowering time differences. The Vigour18 parent was on average 31 cm taller and earlier maturing than 'CM18' (Table 1). Differences in flowering were assessed at ear emergence and recorded using the Zadoks scale (data not shown) (Zadoks et al. 1974). The CM18 parent reached anthesis approximately 10 days later than 'Vigour18'. Leaf width and coleoptile length were weakly correlated with final height but were not associated with differences in time of anthesis (Table 2).

Table 2 Correlation coefficients between mean values of traits leaf width, coleoptile length, height and maturity

	Leaf width 05	Coleoptile length 05	Height field 05	Maturity field 05
Coleoptile length 2004	0.08 (NS)	0.37**	0.22**	0.09 (NS)
Coleoptile length 2005	0.04 (NS)	–	0.17*	0.05 (NS)
Leaf width 2004	0.6**	0.01 (NS)	0.1 (NS)	0.07 (NS)
Leaf width 2005	–	0.1 (NS)	0.19*	0.03 (NS)

** and * indicate significance of Pearson correlation coefficient at $P < 0.01$, 0.05, respectively

Identification of QTLs for leaf width, coleoptile length and height

A framework map was constructed with a total of 244 SSR markers which were mapped to 31 linkage groups representing most of the chromosomal regions of the wheat genome (Fig. 1). Average marker intervals across 21 chromosomes ranged from 6 to 24 cM, covering a total genetic distance of approximately 3,150 cM. The map was used to identify significant associations between markers and width of leaf 3, coleoptile length and final plant height. Using three phenotypic data sets from lines grown in wooden trays and in the field, a region on the short arm of chromosome 6A was associated with an increase in leaf width (Fig. 3). The Vigour18 allele of the SSR marker *NW3106* (221 bp) explained 6–14% of the phenotypic variation for leaf width depending on the environment in which lines were grown (Table 3). Allelic variation at the *NW3106* locus could only account for 6% of the phenotypic variation in field grown lines. Unlike the seed which was grown in trays, field planted seed was not corrected for size, hence variation in seed size and possibly other environmental effects may have reduced the phenotypic variation accounted for by this QTL. A QTL explaining less variation for leaf width was identified on chromosome 2AS with the low vigour parent CM18 contributing the positive effect. The nearest marker *NW2540* accounted for 5–6% of the phenotypic variation in experiments conducted under controlled conditions only.

The 6AS region from 'Vigour18' was also associated with increased coleoptile length. The confidence intervals of the coleoptile length QTL overlapped with the QTL identified for leaf width, though the strongest effect was

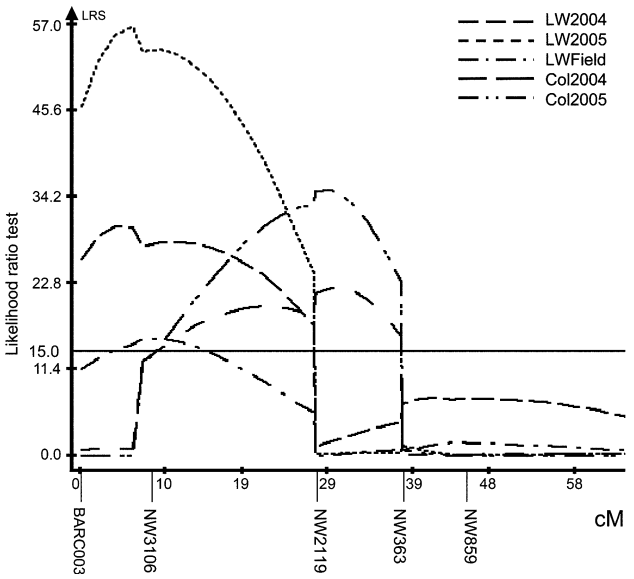

Fig. 3 QTL region on the short arm of chromosome 6A associated with increased leaf width, coleoptile length and final height. A partial linkage group is shown along the *x* axis showing SSR markers and genetic distances (cM). Significance threshold was calculated for all QTLs and the maximum level of 15 ($P = 0.05$) was included. *LW2004* and *LW2005* corresponds to leaf width 3 measurements of RILs grown in trays in 2004 and 2005, respectively. *Col2004* and *Col2005* refer to coleoptile length measurements of RILs grown in trays in 2004 and 2005, respectively

located proximal (Fig. 3). The QTL accounted for 7–8% of the phenotypic variation despite coleoptile length showing relatively low repeatability in this study ($r = 0.37$, $P < 0.01$). Another region on chromosome 3AS was also associated with increased coleoptile length, together with the 6AS QTL accounting for up to 12% of the phenotypic variation. A greater proportion of phenotypic variation was explained in the 2005 experiment compared with 2004 for leaf width and to a lesser extent for coleoptile length. Since a F7 population was scored in 2005 as compared to a F6 generation in 2004, an increase in homozygosity amongst RILs was expected to reduce variation within lines and possibly increase the proportion of explained variation in the F7 population.

Values for final plant height were normally distributed suggesting multiple genes controlling height in this population (Fig. 2). The 6AS region from 'Vigour18' was also associated with increased height in both environments explaining 11–16% of the phenotypic variation. Another smaller height QTL was identified in one of the field sites on chromosome 2DS corresponding to the location of the known dwarfing gene *Rht8* present in 'CM18' (Ellis et al. 2005). The 2DS region was not associated with changes in leaf width or coleoptile length consistent with previous findings where *Rht8* did not reduce seedling vigour (Ellis et al. 2004). A region on chromosome 6AS was identified in 'Vigour18' which promoted coleoptile length and leaf width in early developmental stages but was also associated with greater plant height at maturity.

Determining the effect of 6AS QTL region on leaf width and coleoptile length in other genetic backgrounds

Before QTLs and markers are deployed in marker-assisted breeding, the QTL effect and marker/trait associations require confirmation in other genetic backgrounds.

Table 3 Location of QTLs for leaf width, coleoptile length and plant height in recombinant inbred population derived from a cross between 'Chuan-Mai18' and high vigour breeding line 'Vigour18'

Trait	Trial	Chromosome	Nearest marker	Likelihood[a] ratio test	Variance[b] (%)	Additive effect[c] (mm)
Leaf 3 width	Tray 2004	6AS	NW3106	30	9	0.3
		2AS	NW2540	25	6	–0.25
	Tray 2005	6AS	NW3106	56	14	0.27
		2AS	NW2540	27	5	–0.18
	Field 2005	6AS	NW3106	15	6	0.22
Coleoptile length	Tray 2004	6AS	NW3106	22	7	3.5
		3AS	NW1574	15	4	2.7
	Tray 2005	6AS	NW3106	35	8	3.9
		3AS	NW1574	17	3	2.5
Plant height	Griffith	6AS	NW3106	31	16	53
	2005	2DS	NW1223	29	11	43
	Gundi 2005	6AS	NW3106	20	11	72

[a] Maximum significance threshold of 15 ($P = 0.05$) was determined for Likelihood ratio test statistic using permutation test for above QTLs

[b] Percentage phenotypic variation explained by each marker

[c] Additive effect, negative value indicates that CM18 allele contributed growth promoting effect

Polymorphic markers in experimental populations must distinguish the donor allele from other recurrent background alleles used in the breeding program. In a F3 breeding population which segregated for leaf width and which was derived from a cross between 'Vigour18' and a breeding line Janz 8-28, the SSR marker *NW3106*, which was closest to the leaf width QTL on chromosome 6AS in the experimental population, amplified a 221 bp product from 'Vigour18' and a 233 bp product from 'Janz 8-28'. The subset of lines carrying the Vigour18 allele at the homozygous level had significantly wider leaf 3 than lines with the alternate allele (6.8 ± 0.07 mm vs. 6.4 ± 0.04 mm, $P < 0.01$) (Fig. 4a). These results demonstrated that the QTL on chromosome 6AS of 'Vigour18' accounted for a significant increase in leaf width within an unrelated genetic background.

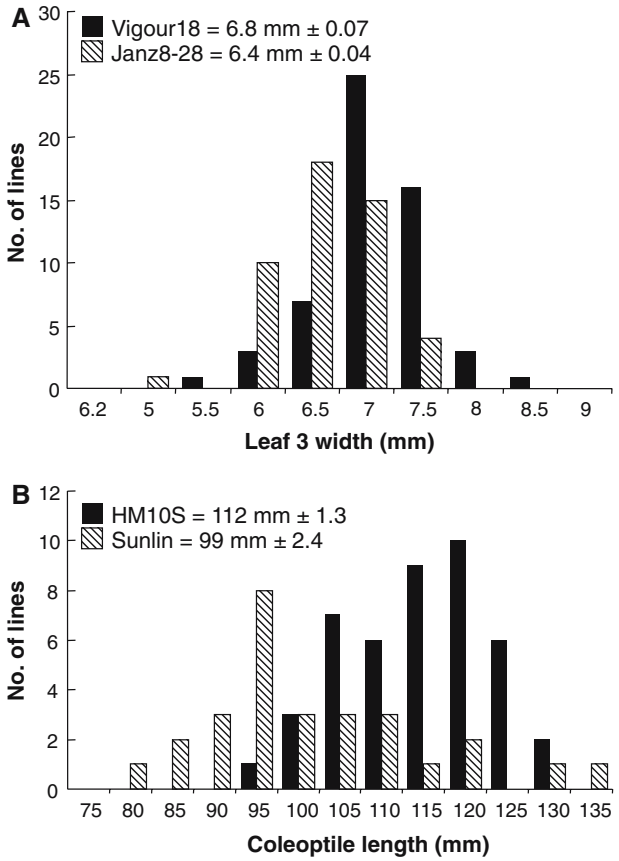

Fig. 4 **a** Frequency distribution of F3 lines from a cross between 'Vigour18' and 'Janz8–28'. Mean leaf 3 width of lines carrying the 'Vigour18' allele of SSR marker NW3106 was significantly greater (6.8 ± 0.07 mm, $P < 0.01$) than mean leaf 3 width of lines carrying the 'Janz8-28' allele (6.4 ± 0.04 mm). **b** Frequency distribution of F6 lines derived from a cross between 'HM10S' and 'Sunlin'. Mean coleoptile length of lines with the 'HM10S' allele of NW3106 marker was significantly longer (112 ± 1.3 mm, $P < 0.01$) than lines with 'Sunlin' allele (99 ± 2.4 mm)

Given that markers from the 6AS region were associated with an increase in leaf width and coleoptile length in the CM18 × Vig18 population, we evaluated 6AS markers in a second breeding population which was segregating for coleoptile length. This population was developed from a backcross between the long coleoptile donor line 'HM10S' and the Australian cultivar 'Sunlin'. The *NW3106* marker which was separated from the QTL peak for coleoptile length by approximately 20 cM in the CM18 × Vig18 population, amplified a 233 bp product from 'HM10S' and 221 bp product from 'Sunlin'. The marker *NW2119* nearest to the QTL for coleoptile length in the CM18 × Vig18 population was monomorphic between parental lines 'HM10S' and 'Sunlin'. On average the HM10S allele of *NW3106* in the homozygous state was associated with longer coleoptiles (112 ± 1.3 mm vs. 99 ± 2.4 mm, $P < 0.01$) demonstrating that a region on chromosome 6AS from two unrelated donor lines contributed to coleoptile length in different genetic backgrounds (Fig. 4b). Because line 'HM10S' was selected from a cross between varieties 'Mara' and 'Halberd', we determined which parent contributed the 233 bp allele. NW3106 marker product in 'HM10S' was the same size as in 'Mara' (233 bp) which varied from the product amplified from 'Halberd' by 12 bp (221 bp) (data not shown).

Discussion

The width of seedling leaves is highly correlated with leaf area and a good predictor of early seedling vigour. To select for increased vigour in wheat breeding programs, taking leaf width measurements was proposed as a simple, non-destructive method to screen germplasm (Rebetzke and Richards 1999). The method relies on good quality seed which needs to be standardized for weight and requires replicated experiments to grow seedlings for 4–6 weeks under cool conditions and natural light before leaf measurements can be taken. Screening with molecular markers linked to QTLs that contribute to increased vigour may complement the phenotype-based selection methodology and lead to more efficient resource allocation within a breeding program.

We have used 'Vigour18', which was developed as a donor line for increased vigour, to identify a region on chromosome 6AS which was associated with increased leaf width and coleoptile length. Although QTLs for leaf width and coleoptile length were overlapping, the genetic intervals corresponding to the strongest association were not aligned. Marker *NW3106* nearest to the leaf width QTL marked one boundary of the coleoptile length QTL, similarly marker *NW2119* nearest to the QTL for coleoptile length marked the boundary of the leaf width QTL. It is

therefore possible that two separate but linked QTLs are located in this region. The relative small effect of these linked QTLs and strong environmental influences probably explain why the phenotypic values of leaf width and coleoptile length were not significantly correlated.

The same region on 6AS contributed to increased height at maturity, indicating that this region may promote basic processes which play a role during early and late stages of plant development. The widely used dwarfing genes *RhtB1b* and *RhtD1b* also affect the same traits, but are associated with reduced coleoptile length, leaf width and plant height. These genes confer insensitivity to endogenous gibberellins and are associated with smaller cell size in dwarf plants compared to tall wheats (Allan et al. 1962; Keyes et al. 1989). It is possible that the QTL on chromosome 6A in 'Vigour18' may have the opposite effect by increasing the average cell size which in turn could contribute to wider leaves. A previous study, however, which compared the size of epidermal cells in seedling leaves of 'Vigour18' with 'CM18' found that increased leaf width in 'Vigour18' was associated with increased number of cell files and not larger cells, therefore discounting the possibility that increased cell size is a major contributor to increased leaf width in 'Vigour18' (Botwright et al. 2005). Early detailed microscopic studies of the wheat embryo suggest that within the embryo most cell files of leaf 1 are present in its primordia. However, leaf 2 and 3 primordia are considerably smaller and unlikely to contain the full complement of cell files present in the mature leaves (Williams 1960). The number of cell files for leaf 1 may be set in the previous generation when the embryo is formed and simply grows out to its predetermined width, whereas with leaf 2 and 3 it is likely that additional cell files are added during germination to reach the final leaf width. Genotypic differences for leaf width may therefore involve processes influencing the rate of cell division during the early stages of seedling growth. A greater number of cell files present in the leaf primordia of 'Vigour18' and the size of the apical meristem may also contribute to increased leaf width. To investigate this further, we are currently comparing cell file numbers in leaf primordia during the germination process between vigorous and non-vigorous wheats.

An important aspect of this study was to go beyond the initial QTL/trait association identified within an experimental population developed from a cross between a Chinese cultivar 'Chuan Mai 18' and a breeding line 'Vigour18' wih exceptional early vigour but poor agronomic characteristics. Because 'Vigour18' was used as a donor line for increased vigour in breeding, we showed that the 221 bp 'Vigour18' allele of marker *NW3106*, which was linked to the QTL for leaf width in the experimental population, also explained a significant proportion of variation in a F3 breeding population. The mean leaf width of F3 lines with the 221 bp allele increased by 0.4 mm which is comparable to the variation in leaf width that exists amongst current Australian wheat cultivars (Rebetzke and Richards 1999).

In a second breeding population (HM10S × Sunlin), the marker *NW3106* also explained a significant proportion of variation for increased coleoptile length. However, in this population the 233 bp allele from HM10S accounted for a 12% increase in coleoptile length in the homozygous state suggesting that there is no linkage disequilibrium between this marker and the coleoptile length QTL amongst parental lines used in this study. Linkage disequilibrium may be limited because of the large genetic distance involved; the marker was approx 20 cM away from the QTL peak in the experimental CM18 × Vig18 population. The HM10S parental line was a selection from a cross between the long coleoptile cultivar 'Halberd' (221 bp allele of *NW3106*) and 'Mara' carrying the 223 bp allele. Based on the phenotype of parental lines, we would have expected the 'Halberd' allele to be associated with coleoptile length unless during the development of 'HM10S' the 221 bp allele was separated from the QTL by recombination. Alternatively, 'Mara' contributed the QTL despite having a shorter coleoptile than 'Halberd' confirming the low level of linkage disequilibrium between this marker and the QTL. Future work will focus on defining QTL locations more accurately and developing more markers that will be tested across a wider range of germplasm of known phenotype. This result showed that two unrelated long coleoptile donor lines (co-ancestry estimates of parental lines were low) carried growth promoting alleles in the 6AS region and that the marker *NW3106* was useful to select lines with increased coleoptile length in different genetic backgrounds.

A backcross strategy at CSIRO Plant Industry uses 'Vigour18' and 'HM10S' as donor lines to develop adapted germplasm with longer coleoptiles and increased vigour. Using markers from the 6AS region will eliminate the recurrent parent alleles from early generations thereby ensuring that only lines with at least one growth promoting allele are being advanced to the next generation. This strategy will increase the frequency of vigour alleles and will reduce the number of lines in subsequent generations freeing up resources within the breeding program. These markers may be useful in other breeding programs which aim to improve early vigour providing the high vigour/long coleoptile QTLs are segregating and the linkage phase to the marker is known in parental lines. Because of the height promoting effect, it is necessary to combine the early vigour QTL region with alternative dwarfing genes such as *Rht8* which reduce plant height and increase harvest index but have no negative effect on early crop growth (Ellis et al. 2004).

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