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QTL Analysis of Plant Height in Hexaploid Wheat Doubled Haploid Population

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Abstract

Plant height and coleoptile length are important determinants of plant yield, however their interaction is not well understood. QTL (Quantitative Trait Loci) mapping of plant height was investigated in a doubled haploid (DH) wheat population so that the effects of loci that control plant height could be compared with those on coleoptile length and yield, in subsequent research. The Diversity Array Technology (DArT) approach was employed for genotyping the population. Five QTL, on chromosomes 2B, 4B, 5B, 6B and 7B, were identified that explained 80% of the observed variation in plant height. The QTLs on chromosomes 2B and 4B were highly significant ($P<0.001$) explaining 16% and 33%, of the observed variation in plant height across seasons, respectively. Marker regression analysis revealed a close association between plant height and DArT marker loci wPt-0335 and wPt-9747. It can be concluded that in this population plant height is under polygenic control. The QTLs that control plant height in a hexaploid wheat population and markers that are associated with these loci may be applied to increase the efficiency of breeding efforts that aim to generate wheats with desired height.

Keywords: Doubled haploid (DH), DArT, QTL mapping, plant height

Introduction

Plant height and coleoptile length are important determinants of wheat grain yield. The two characters are associated in wheats that differ for the major dwarfing genes such as the gibberellic acid insensitive (*gai*) *Rht*.genes (Trethowan et al., 2001). However, the relationship between plant height and coleoptile length is not well characterized in non *Rht* (tall) wheats. It has been identified that tall and semi-dwarf lines exhibit better yields across a range of environments and have larger seed size and longer coleoptiles than the shorter semi-dwarf lines (Trethowan et al., 2001; Chapman et al., 2007). Furthermore, the effect of different loci that control plant height, on coleoptile length, and yield is largely unknown. In order to investigate this relationship QTL mapping of plant height and the comparison of the effects of these loci on coleoptile length and yield must be performed. Investigation into the relationship between plant height, coleoptile length and yield in this population, and the impact of identified QTL on each of these characters will provide valuable information to wheat breeders. This study aims to identify the QTLs that control plant height in a hexaploid wheat population and to identify markers that are associated with these loci.

Materials and Methods

Plant Materials

A synthetic wheat was hybridized with Australian wheat cultivar Lang (QT3765/Sunco) and a doubled haploid population was generated from the hybrid. A tall line (VAW08-A17) from this population was hybridized with a semi-dwarf soft wheat cultivar QALBis(Tincurrin*4/3/Lance *2/Condor*4/3Ag14/14/Tatiara*3//Cook*5VPM1) and a doubled haploid population was generated from the hybrid. The wheat x maize system was applied to generate doubled haploid populations

(Ahmed pers. comm.; Johnson et al., 2005) at the University of Sydney Plant Breeding Institute, Cobbitty, NSW, Australia. The population was grown at The University of Sydney, Plant Breeding Institute sites at Cobbitty and Narrabri in 2005 and 2006, respectively. In 2005 the population was sown in 3m rows. In 2006 the trial design was a RCBD with duplicate 6m rows.

Plant Height Measurement

Plant height of each line was determined by measuring from ground level to the top of the spike at maturity on ten randomly chosen plants for each line, excluding border plants.

DNA Isolation and DArT Marker Analyses

Genomic DNA was extracted from 0.5 to 1 g of fresh leaf tissue (from a bulk of 4 plants of 2 weeks after emergence) according to the CTAB protocol by Doyle et al. 1990. Leaves that were dry, disease free and free from sticking soil particles were selected for DNA extraction. Diversity array technology (DArT) was used in these genetic studies. DNA (2.5*u*g) of each DH line (n=92) and the two parents of the QALBis x VAW08-A17 population were sent to Triticarte Pty. Ltd., Canberra, Australia (http://www.triticarte.com.au) for whole genome profiling (Wenzl et al., 2004; Huttner et al., 2006). The 92 lines that were the subject of this study were randomly selected from the population. The loci were scored as present (1) or absent (0) and the locus designations were as used by Triticarte Pty. Ltd. The P value reflects how well the two phases (present=1 vs absent=0) of the marker are separated in the sample and P is based on ANOVA which is an estimate of marker quality. Markers with P values 80, 77-79, and 75-76 are termed as extremely reliable, and provide useful information, respectively (Wenzl et al. 2004; Huttner et al. 2006). DArT markers consisted of the prefix ‘wPt’, followed by number corresponding to a particular clone in the genomic representation, where w stands for wheat, P for *Pst*I (primary restriction enzyme used) and T for *Taq*I (secondary restriction enzyme) (Wenzl et al., 2004; Huttner et al., 2006).

Linkage Map Construction and QTL Mapping

The initial linkage mapping was performed with Cartablanche software, version 1.5.0(111), Keygene Products B.V. Initially a framework map was constructed using DArT markers. This mapping was continued until acceptable linkage groups were constructed keeping the “tension” level low (preferably below ‘3’). The initial linkage groups were constructed by this software and further reassessed and reconstructed with Map Manager (QTXb20) (Chemelewicz and Manly 2002). In Map Manager, the LOD value indicated the likelihood of linkage by comparing the probabilities of random association of the markers in the progeny to association caused by linkage. The quality of the genetic linkage was assessed repeatedly by looking for crossovers, at map distance, and LOD values. Marker loci order was reassessed using find and report option where necessary. The assignment of markers in linkage groups for individual chromosomes relied on the consensus map of Triticarte Pty Ltd. and ongoing construction of consensus map for the DArT assay (Akbari et al. 2006; Howes pers. comm.; http://www.triticarte.com.au).

Interval mapping was performed at P=0.01 for marker-trait association using Map Manager QTXb20 (Manly et al., 2001). Single marker regression and simple interval mapping tools were used for this purpose. The marker regression function (P=0.01) was performed to find single marker loci linked with the quantitative data. The analysis was carried out for the two different locations/years, and the pooled data from both environments. The LRS thresholds in the regression analyses P<0.01 and P<0.001 were used for declaring significant and highly significant levels, respectively. QTL effects were calculated by using the permutation function. This function provided an approximation to a permutation test according to the method described by Piepho (2001). The parental heights were calculated by comparing means of plant heights of genotypes carrying the positive and negative alleles. The average contribution of each QTL was calculated by comparing means of phenotypic scores of genotypes carrying the positive and negative alleles.

Results

Plant Height Analysis

The plant heights for dwarf parent QALBis and tall parent VAW08-A17 were recorded 86cm and 106cm at Cobbitty, and 69 cm and 84cm at Narrabri respectively. The range between the tallest and shortest lines of the population ranged from 57 to140 cm in 2005 (Cobbitty), and ranged from 39 to105 cm in 2006 (Narrabri). The average plant heights were 98.5 and 74.30 cm for 2005 and 2006, respectively. The distribution appeared to be normal in both years (Figure 1). Transgressive segregation was observed in this population. Plant height of lines within the population were consistent and significantly correlated (r^2=0.59, P<0.001) across years (Figure 2).

DArT Marker Screening

Three hundred and eighty five DArT markers that were polymorphic in the parents were used to construct a genetic map for the QALBis x VAW08-A17 doubled haploid population. The P values of markers ranged from 74.09 to 97.39 with a mean of 84.18 which is extremely reliable (Akbari et al. 2006). The distribution of markers among the genomes were not uniform, viz., 130, 197, and 46 markers were assigned in the A, B and D genomes respectively, and twenty eight markers were unassigned. The call rate ranged from 80.85 to 100 with an average of 92.02. Even genome coverage was achieved except for chromosomes 2D, 3D, 4D, 5D and 6D. DArT analyses did not provide any markers on chromosome 4D.

Linkage Group Construction

A total of 385 polymorphic DArT markers were used to generate a genetic map in the study. Three hundred and nine markers were placed into 30 linkage groups. Markers of the 7B_3 linkage group cosegregated. Seventy six markers could not be assigned to any linkage groups. The total genome coverage was 1728.5cM. Linkage groups could not be formed for chromosomes 3D, 4D and 6D due to insufficient (<3) polymorphic markers. Ten, 13 and 7 linkage groups were generated and assigned to A, B and D genome chromosomes, respectively.

QTL Analyses

The analyses identified five genomic regions that controlled plant height (viz., 2B, 4B, 5B, 6B and 7BS) in both seasons (Table 1). A QTL on chromosome 2B (*QPht.qp-2B*) that was associated with plant height was highly significant in the pooled analysis and significant in each of the seasons (P<0.01, LRS=8.1 and 21 in 2005 and

(a) Cobbitty-2005 (b) Narrabri-2006

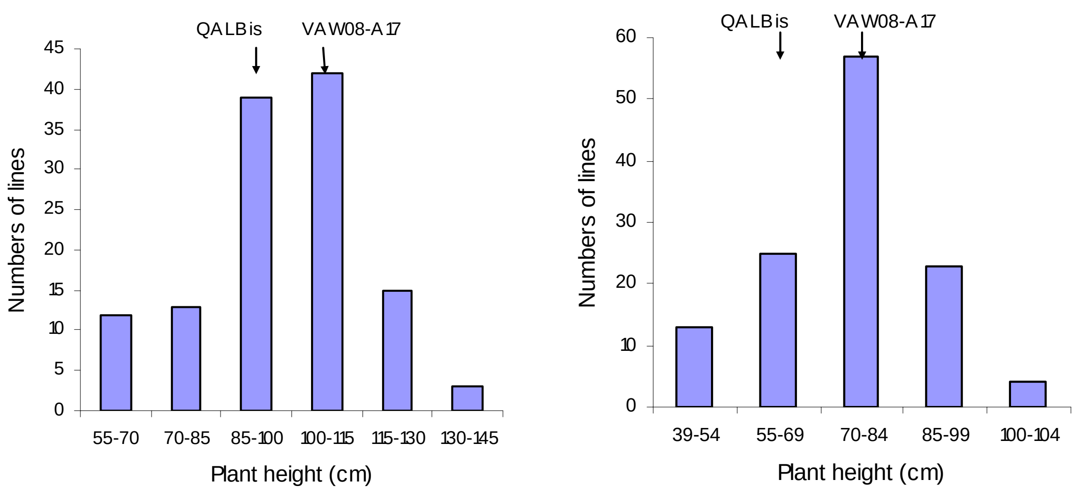

Figure 1 Frequency distributions of plant height (cm) of DH populations derived from QALBis/VAW08-A17 during (a) 2005 and (b) 2006. Arrows indicate parents.

Table 1 Results of interval mapping of Plant height in a set of lines of the QALBis x VAW08-A17 DH population.

Site-Year	Chromosome Location	QTLs	Closest marker	LRS value[1/]	PVE (%)[2/]	Parents (+ve PPO)
Cobbitty-2005	2BS	*QPht.qp-2B*	wPt-0335	8.8*	9	QALBis
	4BS	*QPht.qp-4B*	wPt-9747	25.7**	26	VAW08A17
	5BL	*QPht.qp-5B*	wPt-7561	8.9*	9	VAW08A17
	6BL	*QPht.qp-6B*	wPt-1700	8.7*	9	VAW08A17
	7B	*QPht.qp-7B*	wPt-8920	8.4*	9	VAW08A17
Narrabri-2006	2BS	*QPht.qp-2B*	wPt-0335	21.0**	21	QALBis
	4BS	*QPht.qp-4B*	wPt-9747	31.9**	32	VAW08A17
	5BL	*QPht.qp-5B*	wPt-8449	7.8*	9	VAW08A17
	6BL	*QPht.qp-6B*	wPt-1700	7.5*	9	VAW08A17
	7B	*QPht.qp-7B*	wPt-8920	11.9*	13	VAW08A17
Pooled	2BS	*QPht.qp-2B*	wPt-0335	15.2**	16	QALBis
	4BS	*QPht.qp-4B*	wPt-9747	32.6**	33	VAW08A17
	5BL	*QPht.qp-5B*	wPt-8449	8.6*	9	VAW08A17
	6BL	*QPht.qp-6B*	wPt-1700	7.5*	9	VAW08A17
	7B	*QPht.qp-7B*	wPt-8920	10.8*	13	VAW08A17

[1/] Likelihood statistic ratio; * and ** indicate significant and highly significant level at P<0.01 and P<0.001, respectively. Non asterisk LRS values indicate the suggestive level (P>0.05).
[2/] Phenotypic variation explained.

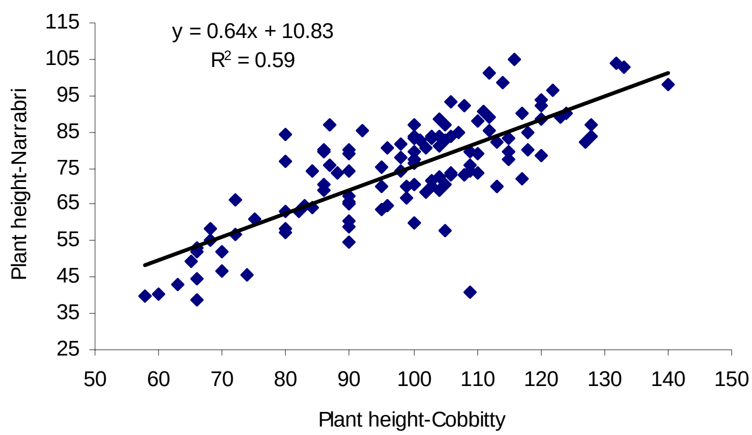

Figure 2 Relationship between plant heights (cm) of lines from the QALBis x VAW08-A17 doubled haploid population in 2005 and 2006 when grown in Cobbitty and Narrabri, respectively.

2006, respectively.). This QTL explained 9 and 19% of phenotypic variation in plant height in 2005 and 2006, respectively. Across seasons the QTL (LRS=15.2) explained 16% of phenotypic variation in height (Figure 3). The QTL (*QPht.qp-4B)* that was most significantly associated with plant height (P<0.001, LRS=25.7 and 31.9 in 2005 and 2006, respectively) was located on the short arm of chromosome 4B. This QTL explained 26 and 32% of variation in plant height in 2005 and 2006, respectively. Pooled analysis revealed that this QTL explained 26% of variation in height (LRS= 32.6) (Figure 3). QTL on chromosomes 5B (*QPht.qp-5B)*, 6B (*QPht.qp-6B*) and 7B(*QPht.qp*-7B) were significantly associated with plant height in 2005, 2006 and across seasons.

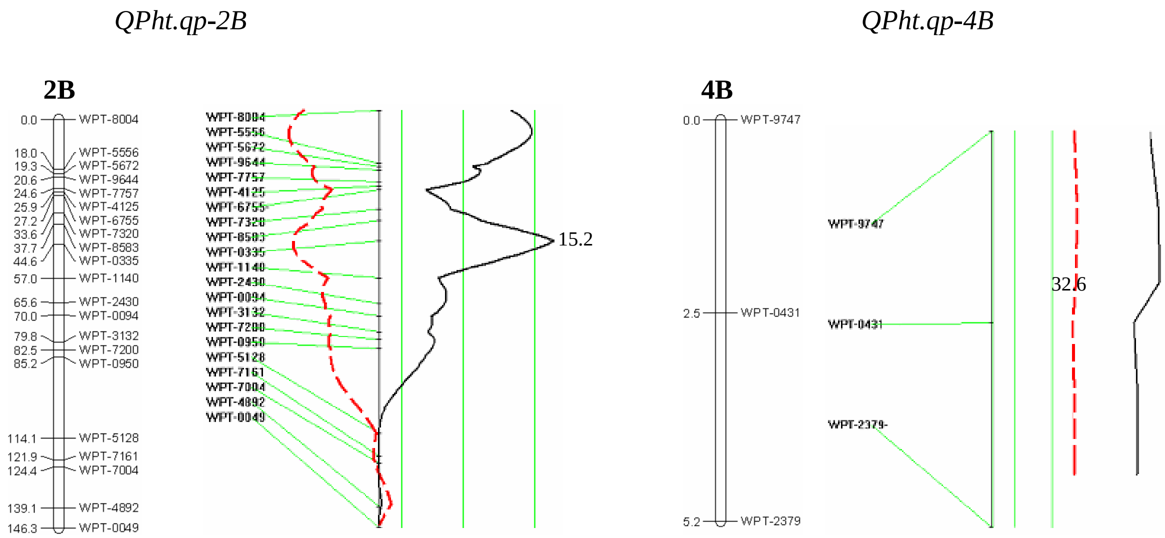

Figure 3 QTLs locations of genomic regions controlling Plant height genes responded on the DH population QALBis/VAW08A17 derived map. Data obtained from two seasons across environments. DArT markers wPt-0335 and wPt-9747 are closely linked with QTLs on chromosomes 2B and 4B respectively. LRS scores and additive effect curves (solid and broken respectively) for interval mapping are placed on the right of the partial map of each chromosome. Vertical lines from left to right indicate suggestive, significant and highly significant levels of LRS values, respectively (determined in 5 cM for 1000 permutations). LRS scores are shown near peaks.

They each accounted for between 9% and 13% and had LRS values between 7.5 and 11.9, in each of the seasons analysed and across (pooled) seasons (Table 1).

Positive additive effects were recorded for the four QTLs on chromosomes 4B, 5B, 6B and 7B in both environments indicating that VAW08-A17 contributed alleles that increased plant height at these loci. A positive additive effect for the QTL on chromosome 2B was observed indicating that the allele conferring increasing plant height was contributed by QALBis.

Discussion

The result agrees with previous findings that chromosomes 2B (Ahmed et al. 2000; Ellis et al. 2005) and 4B (Cadalen et al. 1998; Ahmed et al. 2000) control plant height. *Rht-B1* is located on the short arm of chromosomes 4B (Cadalen et al., 1998) and probably corresponds with the QTL identified in this study. The results obtained in this research indicate that the positive allele of the QTL for plant height reduction on chromosome 4B was contributed by QalBis and it can be suggested that it probably possesses the *Rht-B1* gene. However, QalBis also possessed the allele of the 2B QTL that enhanced height. QTL on chromosome 7B that control plant height have also been identified in other studies (Cadalen et al. (1998) and Ellis et al. (2005). It is not known if they are the same loci. The other two QTLs identified in this study were located on the long arms of chromosomes 5B and 6B. Cadalen et al. 1998 detected QTLs with some other loci related with *Rht* gene. But they were suspicious of the authenticity of these findings and suggested that their result could be either genotype X environment interactions or statistical artifacts. In this investigation both QTLs on chromosomes 5B and 6B appeared across environment with significant LRS values (8.6 and 7.5 respectively) and both accounting for 9% of variation in plant height. This results confirms that minor genes that control this trait are present in wheat. The closely linked DArT marker loci identified for the QTLs would be useful to complement other marker systems as diagnostic tools in Australian cultivars. The application of DArT markers may be limited by the availability of polymorphic markers in some regions (e.g. chromosomes 4B and 7B) due to poor marker coverage. Genetic associations of DArT markers with QTLs on chromosomes 2B, 4B, 5B, and 7B would be useful to identify PCR-based markers for tracing these genes in diverse wheat germplasm. Despite this limitation, the genetic information and marker trait associations detected using Diversity Array Technology will complement other studies in this area.

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