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Increase in coleoptile length and establishment by *Lcol-A1*, a genetic locus with major effect in wheat

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Abstract

Background: Good establishment is important for rapid leaf area development in wheat crops. Poor establishment results in fewer, later-emerging plants, reduced leaf area and tiller number. In addition, poorly established crops suffer from increased soil moisture loss through evaporation and greater competition from weeds while fewer spikes are produced which can reduce grain yield. By protecting the emerging first leaf, the coleoptile is critical for achieving good establishment, and its length and interaction with soil physical properties determine the ability of a cultivar to emerge from depth.

Results: Here we characterise a locus on chromosome 1AS, that increases coleoptile length in wheat, which we designate as *Lcol-A1*. We identified *Lcol-A1* by bulked-segregant analysis and used a Halberd-derived population to fine map the gene to a 2 cM region, equivalent to 7 Mb on the IWGSC genome reference sequence of Chinese Spring (RefSeqv1.0). By sowing recently released cultivars and near-isogenic lines in the field at both conventional and deep sowing depths, we confirmed that *Lcol-A1* was associated with increased emergence from depth in the presence and absence of conventional dwarfing genes. Flanking markers *WB58229* and *IWA710* were developed to assist breeders to select for long coleoptile wheats.

Conclusions: Increased coleoptile length is sought in many global wheat production areas to improve crop emergence. The identification of the gene *Lcol-A1*, together with tools to allow wheat breeders to track the gene, will enable improvements to be made for this important trait.

Keywords: *Triticum aestivum*, Wheat, Coleoptile, Emergence, Molecular marker, SNP

Background

In water-limited environments with high evaporative demand, wheat seedlings need to emerge and develop leaf area rapidly to allow good establishment. Poor establishment reduces the number of spikes per square meter and grain yield [32, 37]. By protecting the emerging first leaf, the coleoptile is critical for achieving good establishment, and its length and interaction with soil physical properties determine the ability of a cultivar to emerge from depth [11, 12, 30].

Longer coleoptiles are beneficial in many agricultural systems worldwide, particularly where deep sowing into

moisture is required [20, 38, 44], where stubble retention practices have been adopted [34] or where crops are sown early into warmer soils [7, 36]. In Australia, there exists a narrow period during which crops must flower in order for yields to be maximised [18], and crop yield is thus very sensitive to timing of establishment. There is an emerging trend to sow crops earlier into warmer soils to optimise whole-farm logistics and increase water-use efficiency [16, 17, 19, 28], but seed-bed soil water potentials are often sub-optimal and temperatures supra-optimal for germination and emergence at this time. The lack of surface moisture during the short, optimum sowing window encourages farmers to sow deep, but if the coleoptile is short, the first leaf may not emerge or may be damaged, leading to poor establishment [38, 44].

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Many current cultivars are not be suited to deep sowing due to their short coleoptiles, and warm soils exacerbate this by further shortening coleoptile length [36]. Long coleoptile wheats would provide growers with more flexibility to sow deep, but at present a lack of knowledge about genes that promote coleoptile growth and efficient selection tools prevent breeders from incorporating this important trait.

Most semi-dwarf cultivars grown worldwide have relatively short coleoptiles due to the presence of dwarfing genes *Rht-B1b* and *Rht-D1b* [23]. These genes encode mutant DELLA proteins that are negative regulators of growth with a large negative effect on coleoptile length which is associated with poor establishment in the field [1, 2, 15, 42]. Despite this, several studies have found variation for coleoptile length within semi-dwarf wheats, suggesting that loci which increase coleoptile length can be selected for in semi-dwarf backgrounds [1, 5].

The search for genetic variation of increased coleoptile length has progressed from phenotypic screening to genome-wide approaches including QTL mapping and genome-wide association studies (GWAS). Preliminary QTL studies focussed on interval mapping approaches in bi-parental populations with sparse maps constructed using AFLP or microsatellite markers [27, 31, 33, 40, 41, 45]. This approach identified multiple QTL with generally small effect (explaining < 5% of phenotypic variation) and large confidence intervals. The advent of cost-effective high density SNP markers and more complex population structures has enabled greater mapping resolution. Rebetzke et al. [35], using a 4-way multi-parent advanced generation intercross (MAGIC) wheat population genotyped with the 9 K SNP array [10], identified seven QTL, while GWAS on a set of 893 wheat accessions also genotyped with the 9 K SNP array identified eight QTL for coleoptile length [26]. Both mapping studies identified QTL on either or both of chromosomes 4B and 4D associated with the dwarfing genes *Rht-B1b* and *Rht-D1b*, confirming the negative effect of these genes on coleoptile length, but also identified loci independent of the dwarfing genes which increased coleoptile length.

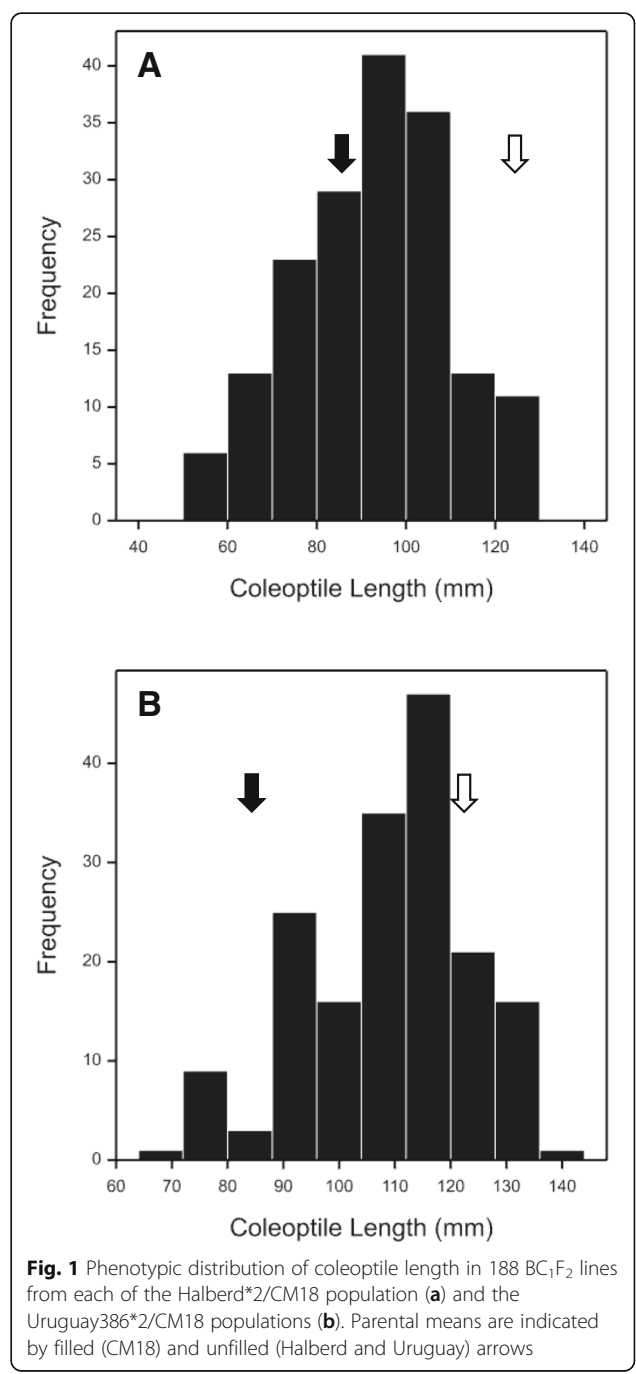
Previous studies highlight that coleoptile length is controlled by many genes with relatively small effects. This study reports the identification, fine mapping, and field validation of a coleoptile length-promoting gene on chromosome 1A of bread wheat. We show that the gene promotes coleoptile length in current elite germplasm, and that longer coleoptiles result in better emergence in both tall and semi-dwarf wheat lines when sown deep in the field.

Results

Genomic regions associated with long coleoptiles

Halberd and Uruguay386 are tall wheats known for their long coleoptiles. To identify genomic regions contributing

to the trait, the coleoptile length was measured in BC₁F₂ lines from two backcross-derived populations of Halberd or Uruguay386 to the short coleoptile Chinese wheat line CM18. As expected, Halberd and Uruguay displayed a long coleoptile phenotype and CM18 a short coleoptile phenotype (Fig. 1). Lines with extremely long and short coleoptiles were selected from these populations for bulked-segregant analysis (BSA) using the 9 K wheat SNP array [10]. Marker-trait associations (MTAs) were detected on 13 wheat chromosomes in the Halberd*2/CM18



population, and 8 chromosomes in the Uruguay*2/CM18 population (Additional file 1: Table S1). Because a large number of tightly linked SNP markers on chromosome 1A were strongly associated with the trait in both populations (Table 1), it was decided to genetically map the trait in this region.

Fine-mapping of the 1A long coleoptile region

For genetic mapping of the trait on chromosome 1A, a new backcross-derived population was developed from HI25M (a Halberd derivative) and Young that lack the *Rht-B1b* dwarfing gene. Fifty-four BC₁F₂ plants were genotyped with *IWA164*, a 1A marker selected from the 1A region in the 9 K SNP consensus map [10]. The association between marker *IWA164* and the trait was confirmed after BC₁F₃ families were phenotyped for coleoptile length, with the marker explaining approximately 30 mm of phenotypic variation for coleoptile

length in this population (Fig. 2). Additional SNP-based markers *IWA3374* and *IWA5435* were selected from the 1A consensus map and shown to flank a genetic interval (~ 10 cM) which contained a gene that will be referred to as *Lcol-A1* (Fig. 3).

To refine the map location of *Lcol-A1*, flanking markers *IWA3374* and *IWA5435* were converted to KASP assays and used to screen 900 BC₂F₂ equivalents from the Young*3/HI25M population to identify 204 recombinants between *IWA3374* and *IWA5435*. These recombinants were genotyped with *IWA164* and a subset of 28 lines were selected for phenotyping. To improve phenotyping accuracy, recombination events were fixed by genotyping BC₂F₃ progeny and selecting segregants that were homozygous at flanking marker loci. Coleoptile length was measured on F₄ families derived from homozygous recombinants to map *Lcol-A1* to a 4 cM interval flanked by markers *IWA164* and *IWA5435* (Fig. 3). An additional seven markers were added to the target interval by screening parental lines with the 90 K SNP array [43] and genotyping recombinants with polymorphic markers. In a second round of fine mapping, a further 57 recombinants (from total of 204) were selected based on *IWA164* and *IWA5435* marker genotypes to measure coleoptile length on BC₂F₄ homozygous, recombinant families. Consequently the map location of *Lcol-A1* was refined to the 2 cM marker interval between *IWB58229-IWA710* which corresponded to approximately

Table 1 Significant ($p < 0.0001$) SNPs on chromosome 1A detected from the bulked-segregant analysis (BSA) in the Halberd*2/CM18 and Uruguay386*2/CM18 populations. Locations (_Loc; cM) from the 9 K [10] and 90 K [43] consensus maps are provided. NA = SNP not placed on consensus map

SNP Name	SNP ID	9K_Loc	90K_Loc
Halberd*2/CM18			
w SNP_Ku_c17726_26872129	IWA6636	51.12	71.05
w SNP_Ex_c3253_5995011	IWA3399	54.28	NA
w SNP_Ex_c6826_11774795	IWA4578	57.95	70.10
w SNP_Ku_c3468_6420199	IWA6942	57.95	70.10
w SNP_Ex_c3747_6824863	IWA3612	57.95	70.10
w SNP_Ex_c8364_14095508	IWA4797	62.39	71.10
w SNP_Ku_c37925_46679146	IWA6972	70.70	71.10
w SNP_Ku_c33917_43336069	IWA6934	109.32	101.19
Uruguay386*2/CM18			
w SNP_Ex_c5323_9408829	IWA4163	35.82	51.09
w SNP_Ku_c11896_19337444	IWA6441	39.00	NA
w SNP_BE586140A_Ta_2_1	IWA360	40.65	55.18
w SNP_Ex_c34821_43076533	IWA3499	51.12	71.48
w SNP_Ex_c3253_5995011	IWA3339	54.28	NA
w SNP_Ex_c2749_5091813	IWA3115	56.97	70.10
w SNP_JD_rep_c49359_33578909	IWA6260.1	57.95	70.10
w SNP_Ex_rep_c104050_88861052	IWA5109	57.95	70.10
w SNP_Ra_c26956_36503468	IWA7804	57.95	70.10
w SNP_Ex_c2389_4477096	IWA2847	57.95	70.10
w SNP_Ex_c3142_5808330	IWA3338	64.39	70.10
w SNP_BE518393A_Td_2_3	IWA352	64.39	70.10
w SNP_Ex_rep_c66382_64577768	IWA5226	67.01	70.10
w SNP_CAP11_c1029_611774	IWA639	68.32	70.10
w SNP_Ku_rep_c71909_71634013	IWA7505	68.65	70.79

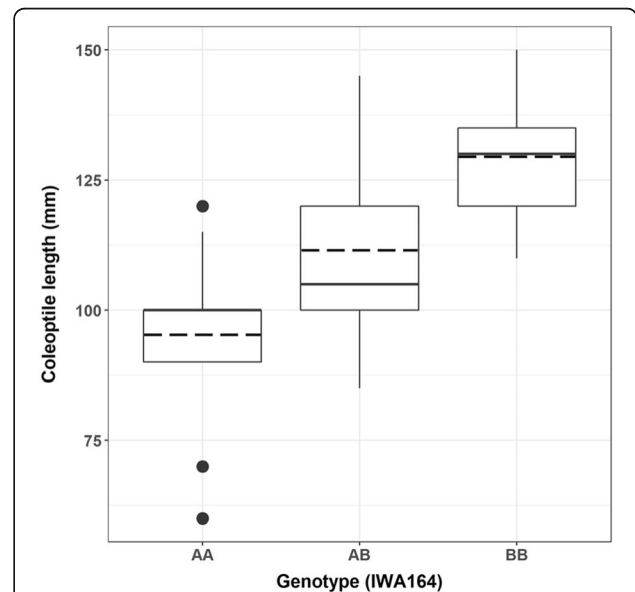
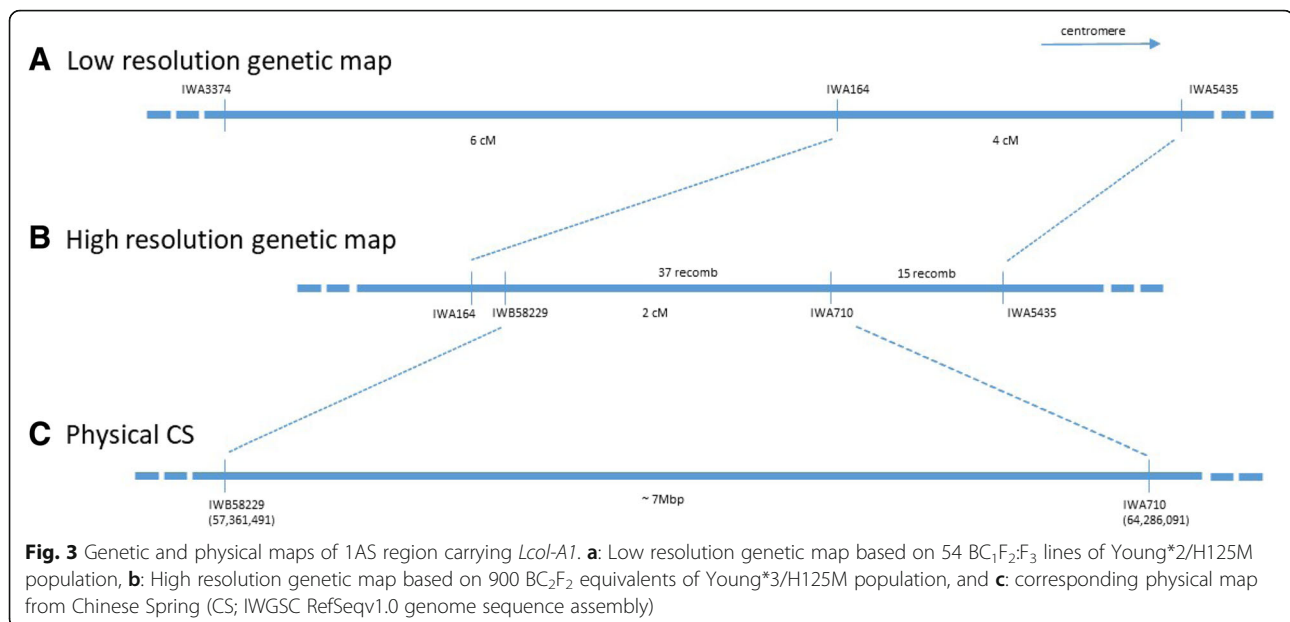


Fig. 2 Association between genotype at marker *IWA164* and coleoptile length of BC₁F₃ seedlings from the Young*2/HI25M. AA, homozygous negative; AB, heterozygous; BB homozygous positive. The lower and upper edges of the box represent 25th and 75th percentiles, interior solid lines denote the median and dashed lines denote the mean. Potential outliers are plotted as black dots, and identified according to the 1.5x interquartile range criterion



7 Mb of physical distance in the RefSeqv1.0 genome sequence assembly [3].

Validation of *Lcol-A1* in Espada genetic background

The effect of *Lcol-A1* was confirmed by transferring the long coleoptile haplotype from HI25M into the Australian cultivar Espada. In a backcross-derived population (Espada*4/HI25M) which lacked the *Rht-D1b* dwarfing gene from Espada, coleoptile length was continuously distributed ranging from 65 to 140 mm in length (Fig. 4). The same marker *IWA164* which was linked to the trait in the Young background explained approximately 25 mm of variation in homozygous lines carrying different haplotypes in the Espada background. These results confirm that *Lcol-A1* contributes to coleoptile growth in another genetic background indicating a major gene for coleoptile growth has been identified.

Lcol-A1 contributes to better emergence in the field

To show that the long coleoptile trait conferred by *Lcol-A1* also improves plant emergence and establishment under field conditions, we tested the hypothesis that cultivars with *Lcol-A1* produce longer coleoptiles and emerge better when sown deep under field conditions compared to cultivars that lack the long coleoptile allele of the gene. Five cultivars that were predicted to carry the long allele based on the haplotype of flanking markers and pedigree and six that were predicted to carry the short allele were selected to test the hypothesis (Table 2). These released cultivars represented a wide range of genetic diversity of the Australian genepool. To determine the effect of *Lcol-A1* in tall background, isolines from the Young*3/HI25M population (five short

and five long haplotype) were also evaluated for emergence under field conditions.

Prior to field testing, we confirmed that *Lcol-A1* contributed to coleoptile length in semi-dwarf cultivars which were included in field trials. The coleoptile length, when measured under controlled conditions, was approx. 18%

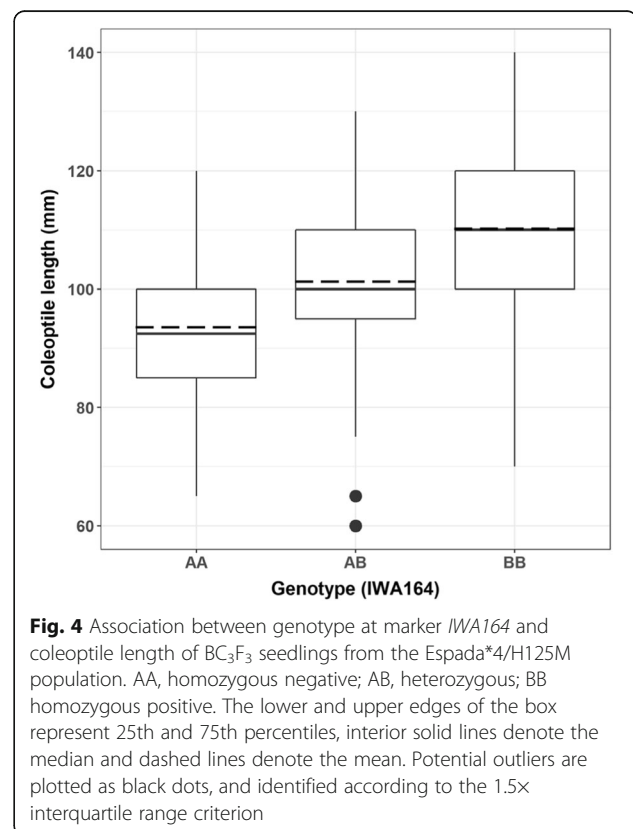


Table 2 Cultivars selected for examining the effect of *Lcol-A1* on emergence in the field. *Lcol-A1* haplotype and year of release (Year) are indicated. Pedigree information was retrieved from the Genetic Resources Information System for Wheat and Triticale (GRIS; wheatpedigree.net)

Cultivar	<i>Lcol-A1</i> Haplotype	Year	Pedigree
EGA Gregory	Short	2004	Pelsart/3*Batavia
Emu Rock	Short	2011	Westonia/Kukri/Perenjori/Ajana
Espada	Short	2008	RAC-875/Krichauff//Excalibur/Kukri/3/RAC-875/Krichauff/4/RAC-875//Excalibur/Kukri
Mace	Short	2008	Wyalkatchem/Stylet//Wyalkatchem
Suntop	Short	2011	Sunco/2*Pastor//SUN-436-E
Young	Short	2005	VPM-1/3*Beulah//Silverstar
Excalibur	Long	1990	RAC-177/Uniculm-492//RAC-311-S
Magenta	Long	2007	Carnamah/Tammin-18
Phantom	Long	2010	Sentinel*3/Yitpi
Scout	Long	2009	Sunstate/QH-76-1//Yitpi
Yitpi	Long	2000	C-8-MMC-8-HMM/Frame

Significant ($p < 0.0001$) SNPs on chromosome 1A detected from the bulked-segregant analysis (BSA) in the Halberd/CM18 and Uruguay386/CM18 backcross populations. Locations (_Loc; cM) from the 9 K [10] and 90 K [43] consensus maps are provided. NA = SNP not placed on consensus map

longer in cultivars predicted to carry the long *Lcol-A1* allele (Fig. 5). In tall isolines the final length increased compared to the cultivars but the difference between genotypes also increased to approximately 25% consistent with an expected growth promotion effect in the absence of conventional dwarfing genes.

For field validation, cultivars were sown at depths of 25 mm and 70 mm representing normal and deep sowing treatments at Yanco in western NSW (Australia) in 2016 and 2017. Emergence was counted in regular intervals during the first 5 weeks of the experiments and the mean emergence counts were plotted for both sowing depths and genotype categories (Fig. 6). In both years, more plants emerged from the deep sowing treatment in

cultivars with the long *Lcol-A1* allele than in cultivars with the short allele. The difference in emergence from deep sowing was more pronounced in the Young*3/HI25M isolines, confirming the positive effect of the gene under field conditions. Interestingly, the increase in emergence afforded by *Lcol-A1* was apparent in cultivars in 2016 and in isolines in both 2016 and 2017 under the normal sowing depth as well.

Prevalence of *Lcol-A1* in an Australian diversity panel

To assess the prevalence of *Lcol-A1* in Australian germplasm, a panel of 197 cultivars and lines that were released from the year 1890 to 2015 and represent a wide range of the Australian gene pool, were

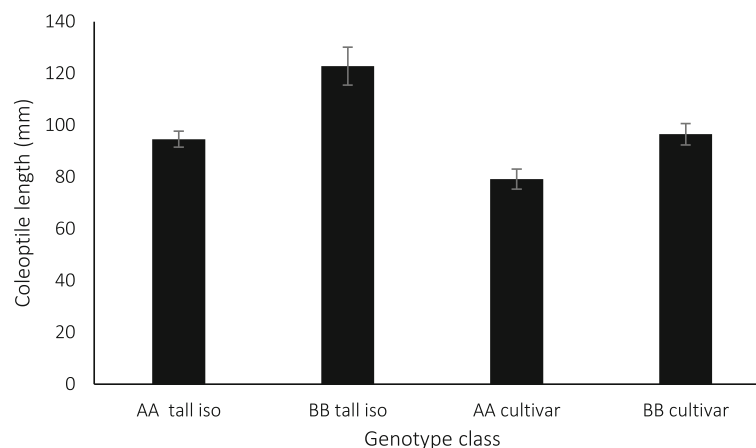
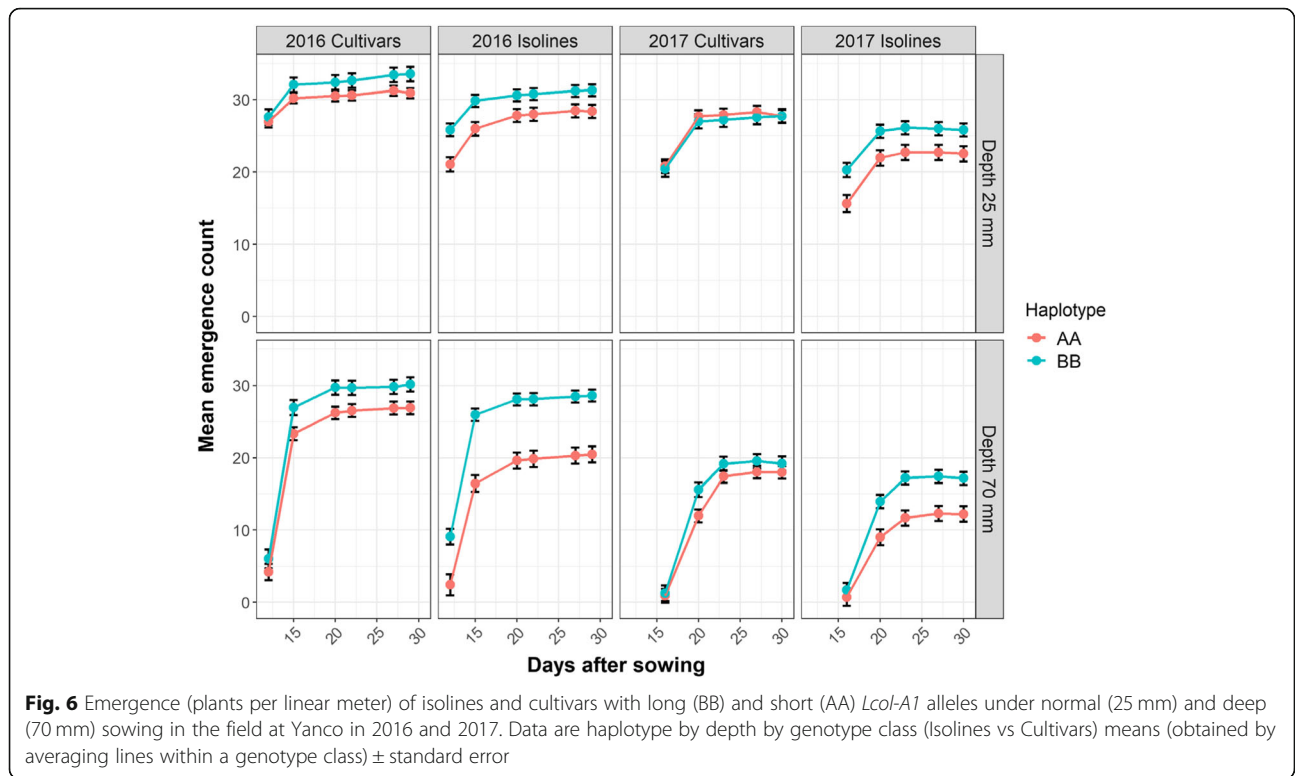
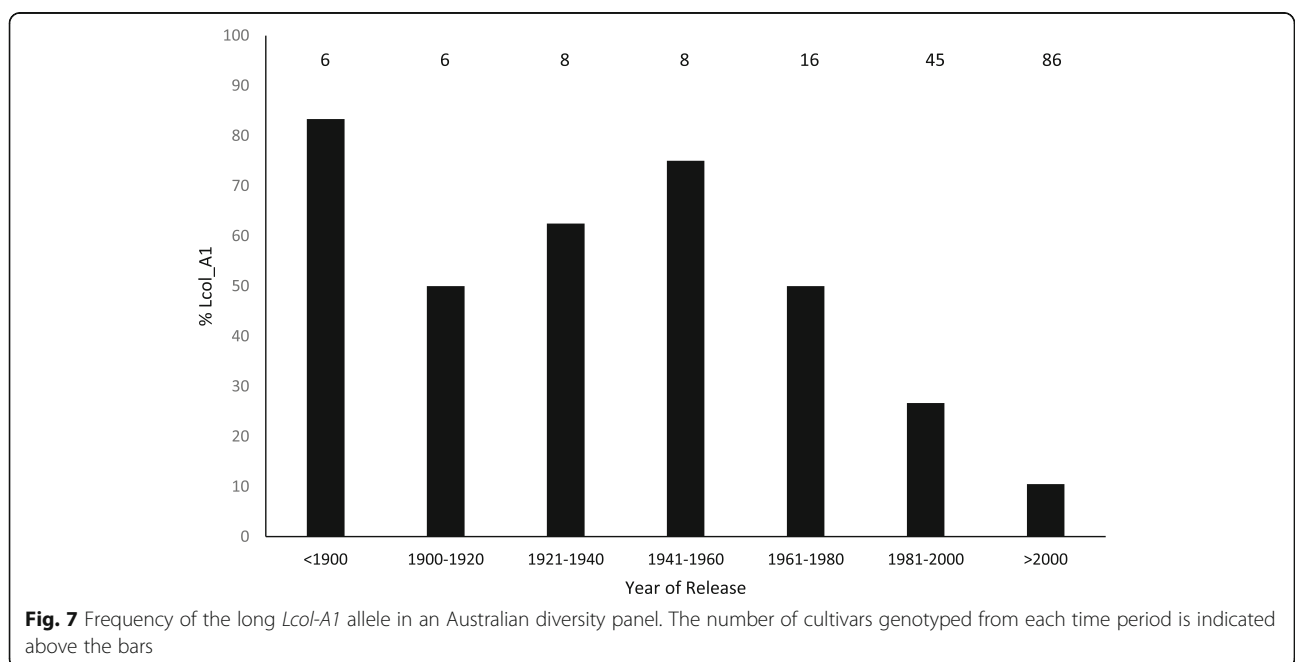


Fig. 5 Coleoptile length of isolines (iso) and cultivars with long (BB) and short (AA) *Lcol-A1* alleles under controlled conditions. Data are means ($n = 5$ for each isoline class; 6 for the short [AA] cultivar class; and 5 for the long [BB] cultivar class, obtained by averaging values from varieties within the relevant [isoline vs cultivar] genotype class) \pm standard error; differences within genotype class are significant ($p = 0.011$ for cultivars, $p < 0.01$ for tall isolines)



screened with markers *IWB58229* and *IWA710*. Across all germplasm, the long *Lcol-A1* containing haplotype was predicted to be present in 24%, absent in 64%, and recombinant in 12% (Additional file 2: Table S2). An assessment of its evolution through the panel suggested that, despite the limited number of

genotypes sampled in the early part of the twentieth century, *Lcol-1A* was reasonably common until 1960, but declined from 1960 to the latest cultivars released in 2015. To this end, the long *Lcol-A1* allele was only present in 10% of the 56 varieties released from 2000 to 2015 (Fig. 7).



Discussion

Good crop establishment presents challenges in many wheat growing regions of the world, and long coleoptile wheats are predicted to help mitigate constraints on emergence imposed by adoption of new management practices and a changing climate. This study characterised *Lcol-A1*, a gene which promotes coleoptile length and which is present in current Australian elite wheat germplasm. The gene is responsible for coleoptile length increases even in the presence of common DELLA dwarfing genes which translated to improved emergence with deep sowing in the field. Tightly linked SNP-based markers have been developed that can be used by breeders to assist in the selection process. Information about the gene and its effect under field conditions together with validated markers delivers a useful package to breeders and the industry and represents a step change from the background-specific marker-trait associations of small effect that were often generated in QTL studies.

A Young*3/H125M population (H125M is a Halberd derivative) was chosen for fine-mapping due to the large effect of *Lcol-A1* and the clear phenotypic difference between homozygous classes. By coupling this genetic material to the fast and simple controlled environment screen used herein, we were able to delineate *Lcol-A1* to a 2 cM region, which corresponded to a physical distance of approximately 7 Mb in the RefSeqv1.0 genome sequence assembly [3]. Work is on-going to identify a candidate gene, prove its function and generate a gene-based marker for accurate selection in breeding programs. In the meantime, the flanking markers reported here will provide an efficient selection tool, particularly when flanking markers are combined to assay the Halberd-derived haplotype.

We showed that *Lcol-A1* was effective in an Espada background, conferring an approximately 25 mm increase in coleoptile length. This validation was important, as it indicated that *Lcol-A1* was effective in other genetic backgrounds additional to those used in the BSA and in the fine-mapping. Further evidence of the robustness of *Lcol-A1* was provided from previous QTL mapping studies. Rebetzke et al. [33] identified a Halberd-derived QTL for coleoptile length on 1AS, in a Cranbrook/Halberd Doubled Haploid population. In a 4-way MAGIC population, the largest effect QTL outside of the common DELLA dwarfing genes *Rht-B1* and *Rht-D1* was located on chromosome 1AS, inherited from cultivar Yitpi [35]. Yitpi and Halberd are related through pedigree, indicating that Yitpi carries *Lcol-A1* by descent. Confirmation of the effect of *Lcol-A1* in these populations provides further evidence of the utility of the gene across different genetic backgrounds.

Under conditions of deep sowing, *Lcol-A1* was associated with an increase in emergence of up to approximately 40% in tall isolines, and of approximately 15% of

cultivars carrying *Rht-B1b* or *Rht-D1b*, confirming that the increase in coleoptile length afforded by *Lcol-A1* translates to improved emergence. The consistent effect of *Lcol-A1* detected in both the controlled environment screen with the field experiments confirms numerous reports about the positive relationship between coleoptile length and field emergence (e.g. [2, 42, 44]). The increased emergence under standard sowing depth with genotypes carrying *Lcol-A1* indicates that the gene may also contribute to a faster rate of coleoptile growth. Other studies also showed that increased coleoptile length is associated with increased rates of emergence [2, 38]. In Western Australia, a delay in sowing reduces yield by 28–36 kg ha⁻¹ day⁻¹ across germplasm with diverse sowing dates and phenology [39]; the increased emergence associated with *Lcol-A1* may translate to reducing these yield losses by reducing the number of later emerging seedlings. Indeed, simulation studies by Kirkegaard and Hunt [25] and Flohr et al. [19] suggest that, when combined with appropriate management strategies, the long coleoptile trait can contribute substantially to farm yield.

Lcol-A1 is present in ~20% of lines that were included in the Australian wheat diversity panel. When assessing the pedigree of Halberd (long coleoptile donor used in this study), it is possible that *Lcol-A1* can be traced back to cultivars Steinwedel (a selection from the 1890's) and Federation (released in Australia in 1901; Additional file 3: Figure S1). Although *Lcol-A1* was reasonably prevalent in Australian wheats until the 1960's, its presence appears to have decreased to only 10% of tested cultivars released since 2000. During the 1960's and 1970's DELLA dwarfing genes were introduced into Australia from CIMMYT, and CIMMYT derived material became dominant parents in Australian breeding programs [8]. None of the Mexican wheats from this era which were assayed with markers carried *Lcol-A1*, and so it is possible that the decline in *Lcol-A1* prevalence is the result of breeders preferring to use parental lines from CIMMYT over the past 50–60 years [24]. Alternatively, it may be that *Lcol-A1* is linked to a trait that Australian wheat breeders are unwittingly selecting against.

Bernardo [6] laments the lack of impact for breeding from the many linkage mapping studies conducted to date, but results from mapping *Lcol-A1* suggests otherwise for this trait. As opposed to novel loci from wild relatives and the challenge of reducing linkage drag when introgressing such loci, the *Lcol-A1* haplotype is present in currently available elite varieties, which should aid in its transfer within breeding programs. Current work is underway to further refine the map location and to isolate the *Lcol-A1* gene which will generate a gene-based marker. In the meantime, tightly linked and flanking SNP markers *IWB58299* and *IWA710* will

be useful to predict the presence of the long allele of *Lcol-A1* in diverse germplasm.

Conclusions

Establishment is a key phase in the life cycle of crops, and the length of the coleoptile significantly impacts upon establishment in wheat. As a result of changes in farming practices to earlier sowing into warmer soils, growers require that wheats with longer coleoptiles that are adapted to these changes are developed. Here, we confirm that *Lcol-A1* increases coleoptile length which results in improved emergence under field conditions. By developing tightly linked flanking markers, we have provided tools to allow the wheat breeding community to incorporate *Lcol-A1* more efficiently in future cultivars.

Methods

Phenotyping for coleoptile length – controlled environment

For coleoptile length measurements, 14 seeds/line were sown in 100 mm deep wooden trays (50:50 fertile compost/vermiculite soil mix) and grown in growth cabinets at 15 °C for 14 days (210 degree days) in the dark before coleoptile lengths were measured with a ruler.

Bulked-segregant analysis

One-hundred and eight-eight (188) BC₁F₂ progeny from a Halberd*2/Chuan-Mai18 (CM18) population (Halberd is an older Australian wheat with a long coleoptile, while CM18 is a Chinese wheat with a short coleoptile [41] and 188 BC₁F₂ progeny from a Uruguay386*2/CM18 population (a tall Uruguay accession (AUS1517) from the Australian Winter Cereals Collection with a long coleoptile) were phenotyped for coleoptile length to identify 10 short and 10 long coleoptile lines from each population. DNA from each class was pooled separately for each population and genotyped by BSA using the 9 K SNP array at Agriculture Victoria Research, La Trobe University [10]. These populations were used for BSA only and not progressed for mapping.

Genetic mapping of the long coleoptile gene

Two backcross populations developed from HI25M (long coleoptile Halberd derivative) and Young (short coleoptile cultivar) were used to map the long coleoptile gene. Firstly, 434 BC₁F₂ plants were screened with a marker derived from the *Rht-B1b* dwarfing allele [13] to identify 54 BC₁F₂ plants that lacked *Rht-B1b*. The elimination of the known dwarfing gene at an early stage resulted in tall progeny with only minor height variation that was derived from this population for fine mapping. These plants were genotyped with marker *IWA164* which was mapped in the 9 K SNP consensus map of wheat [10] to the 1A region identified in BSA, and BC₁F₃ progeny were phenotyped for coleoptile

length under controlled conditions (see above). Similarly, the plants were genotyped with markers *IWA3374* and *IWA5435* from the 9 K SNP consensus map, which flanked the 1A region identified in BSA. The BC₁ derived population was used to map the long coleoptile gene to chromosome 1A and to identify flanking SNP-based markers that were critical for the next mapping step.

To increase the resolution of the target region, BC₂F₃ progeny from BC₂F₂ plants that were heterozygous for markers in the 1A region were screened with flanking markers *IWA3374* and *IWA5435*. DNA was extracted from half-seeds of 900 BC₂F₃ individuals using the protocol of Ellis et al. [14]. To make the screening process more efficient, SNP-based markers were converted to Kompetitive allele specific PCR (KASP) assays [22].

Plants from half seed that carried recombination events between the flanking markers were selfed. Twelve to 16 progeny were genotyped to identify individual plants that were recombinant and homozygous in the target region. Coleoptile length was measured in homozygous progeny which were used to fine map the gene with additional 90 K SNP markers that were polymorphic between Halberd and Young, developed from the consensus map of the region.

Validation of the 1A region

The 1A region was transferred from HI25M to Espada (an Australian cultivar) through backcrossing. BC₃F₃ plants were genotyped for *Rht-D1b* [13], *Rht18* [21], and the 1A locus. Lines that were homozygous wild-type for *Rht-D1*, homozygous dwarf for *Rht18*, and heterozygous for the 1A locus were selfed to produce BC₃F₃ derived near isogenic lines (NILs). Coleoptile length of the NILs was phenotyped using the method described above, and the same plants genotyped to assess marker-trait associations.

Field performance

To validate the 1A locus across different genetic backgrounds and to provide evidence that the increase in coleoptile length improves emergence under field conditions, two field experiments were conducted at Yanco (34°36' 53.6"S 146°24' 54.2"E) in Southern New South Wales, Australia in 2016 and 2017. A split-plot randomised block design balanced for row and column and consisting of four replicates of 10 tall isolines (five short and five long coleoptile haplotype) of BC₁F₅ plants from the Young*3/HI25M population as well as 11 diverse Australian cultivars (six short and five long coleoptile 1A haplotype) were sown at depths of 25 mm and 70 mm (main plots) in plots that were 7 m long and 8 rows wide, with 20 cm row spacing. Emerged plants (plants per linear meter) were counted in four rows per plot 12, 15, 20, 22, 27, and 29 days after sowing in 2016, and 16, 20, 23, 27, and 30 days after sowing in 2017. Sowing depth in the deep sowing treatment was

checked by measuring the coleoptile length in seedlings after they emerged. Cultivars carried either *Rht-B1b* or *Rht-D1b* dwarfing gene and were similar in final plant height. They were chosen based on their adaptation to different climatic zones of the Australian wheat belt.

Allele survey and pedigree assessment

To assess the frequency of the 1A haplotype from Halberd across diverse germplasm, DNA from an Australian diversity panel comprised of 197 bread wheat cultivars and lines was assayed with markers *IWB58229* and *IWA710*. To assess pedigree relationships of lines sharing a common haplotype, the Genetic Resources Information System for Wheat and Triticale (GRIS; wheatpedigree.net) was queried.

Statistical analyses

The controlled environment and the 2016 and 2017 field experiment datasets were analysed using the linear mixed model software *asreml* [9] for R (R [29]).

For the controlled environment experiment, cultivar and isolate genotype class groups were analysed separately. To compare haplotypes, the traditional 'one-way ANOVA' analysis for comparing two treatment groups was extended to a linear mixed model by representing varieties comprising the particular genotype class as a random effects factor in the statistical model, thus ensuring the correct degrees of freedom were used for the haplotype comparison. F-tests for the significance of the haplotype term yield the *p*-values for the comparison between AA and BB haplotypes within each genotype class. Potential outliers were identified using the 1.5x interquartile range criterion [4].

The field experiments in 2016 and 2017 were analysed separately, although isolines and cultivars were analysed together within each trial. The field experiments involve factorial treatment structures for haplotype, genotype class and sowing depth, modelled as fixed effects. Since the haplotype/genotype class combinations represent groups of varieties, variety was included in the model as a nested random effects factor, and variety interaction terms were therefore modelled as random effects terms. Further random effects terms were included to represent the spatial structure of the trial layout, namely block, plot and subplot (representing row within plot). The models incorporated the recording of observations across multiple dates by accounting for autocorrelation structure across the (unevenly spaced) dates, and allowing heterogeneity of the residual variances across dates. For the 2016 dataset the model also allowed for unequal variances that were observed between the two sowing depths.

Additional files

Additional file 1: Table S1. All significant marker-trait associations detected from BSA using the 9 K SNP array in the Halberd*2/CM18 and Uruguay*2/CM18 populations. (DOCX 29 kb)

Additional file 2: Table S2. Genotyping results from the Australian diversity panel. Cultivar, year of release, and allele call (A indicates short allele; B indicates long allele) for markers *IWB58229* and *IWA710* are shown. (DOCX 31 kb)

Additional file 3: Figure S1. Pedigree of Halberd. Cultivars which were genotyped in the diversity panel carrying the long (green) or short (yellow) *Lco1-A1* alleles are highlighted. Gaza, a cultivar carrying a recombinant haplotype is highlighted in orange. (DOCX 270 kb)

Abbreviations

AFLP: Amplified fragment length polymorphism; BSA: Bulk-segregant analysis; GWAS: Genome wide association study; KASP: Kompetitive allele specific PCR; MAGIC: Multi-parent advanced generation intercross; MTA: Marker trait association; QTL: Quantitative trait locus; SNP: Single nucleotide polymorphism

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Authors' contributions

Wrote the manuscript, developed germplasm and performed experiments: WDB. Performed experiments and reviewed the manuscript: JH. Performed statistical analyses and reviewed the manuscript: ABZ. Performed fine-mapping experiments and reviewed the manuscript: BAF. Developed germplasm and reviewed the manuscript: BJB and GJR. Performed experiments: GP and TP. Undertook 9 K SNP BSA analysis and reviewed the manuscript: MJH. Oversaw field experiments and reviewed the manuscript: JRH. Conceived, supervised the experiments, contributed to manuscript writing and revision: WS. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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