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 dwarving genes. Flanking markers IWB58229 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 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 BC$_1$F$_2$ 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 BC 1F2 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 BC 1F3 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 BC_2F2 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 BC_2F3 progeny and selecting segregants that were homozygous at flanking marker loci. Coleoptile length was measured on F_4 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 BC_2F4 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

| SNP Name       | SNP ID     | 9K_Loc | 90K_Loc |
|----------------|------------|--------|---------|
| wsnp_Ku_c17726_26872129 | IWA6636    | 51.12  | 71.05   |
| wsnp_Ex_c3253_5995011  | IWA3399    | 54.28  | NA      |
| wsnp_Ex_c6826_11177495  | IWA4578    | 57.95  | 70.10   |
| wsnp_Ku_c3468_6421099   | IWA6942    | 57.95  | 70.10   |
| wsnp_Ex_c3747_6824863   | IWA3612    | 57.95  | 70.10   |
| wsnp_Ex_c3836_14095508  | IWA4797    | 62.39  | 71.10   |
| wsnp_Ku_c37925_46679146  | IWA6972    | 70.70  | 71.10   |
| wsnp_Ku_c33917_43336069  | IWA6934    | 109.32 | 101.19  |
| wsnp_Ex_c5323_9408829   | IWA4163    | 35.82  | 51.09   |
| wsnp_Ku_c11896_19337444  | IWA6441    | 39.00  | NA      |
| wsnp_BES86140A_Ta_2_1    | IWA360     | 40.65  | 55.18   |
| wsnp_Ex_c34821_43076533  | IWA3499    | 51.12  | 71.48   |
| wsnp_Ex_c3253_5995011    | IWA3339    | 54.28  | NA      |
| wsnp_Ex_c2749_5091813    | IWA3115    | 56.97  | 70.10   |
| wsnp_ID_rep_c49359_35357809  | IWA6260.1  | 57.95  | 70.10   |
| wsnp_Ex_rep_c104050_88861052  | IWA5109    | 57.95  | 70.10   |
| wsnp_Ra_c26056_36503468  | IWA7804    | 57.95  | 70.10   |
| wsnp_Ex_c3289_4477096    | IWA2847    | 57.95  | 70.10   |
| wsnp_Ex_c3142_5808330    | IWA3338    | 64.39  | 70.10   |
| wsnp_BES83933A_Td_2_3    | IWA352     | 64.39  | 70.10   |
| wsnp_Ex_rep_c66382_64577768  | IWA5226    | 67.01  | 70.10   |
| wsnp_CAP11_c1029_611774  | IWA639     | 68.32  | 70.10   |
| wsnp_Ku_rep_c71099_71634013  | IWA7505    | 68.65  | 70.79   |

**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.
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 gene pool. 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%
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 represented a wide range of the Australian gene pool, were

| Cultivar  | Lcol-A1 Haplotype | Year | Pedigree                                                                 |
|----------|-------------------|------|--------------------------------------------------------------------------|
| EGA Gregory | Short            | 2004 | Pelsart/3*Batavia                                                         |
| Emu Rock  | Short            | 2011 | Westonia/Kukri/Perenjori/Ajana                                            |
| Espada    | Short            | 2008 | RAC-875/Kirchaufl/Excalibur/Kukri/3/RAC-875/Kirchaufl/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-HMW/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.
screened with markers IWB58229 and IWA710. Across all germplasm, the long \textit{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, \textit{Lcol-1A} was reasonably common until 1960, but declined from 1960 to the latest cultivars released in 2015. To this end, the long \textit{Lcol-A1} allele was only present in 10% of the 56 varieties released from 2000 to 2015 (Fig. 7).

**Fig. 6** Emergence (plants per linear meter) of isolines and cultivars with long (BB) and short (AA) \textit{Lcol-A1} alleles under normal (25 mm) and deep (70 mm) sowing in the field at Yanco in 2016 and 2017. Data are haplotype by depth by genotype class (Isolines vs Cultivars) means (obtained by averaging lines within a genotype class) ± standard error.

**Fig. 7** Frequency of the long \textit{Lcol-A1} allele in an Australian diversity panel. The number of cultivars genotyped from each time period is indicated above the bars.
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. Rebetzk 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 [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 1890s) and Federation (released in Australia in 1901; Additional file 3: Figure S1). Although Lcol-A1 was reasonably prevalent in Australian wheats until the 1960s, its presence appears to have decreased to only 10% of tested cultivars released since 2000. During the 1960s and 1970s 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.

Bernando [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 IWBS8299 and IWA710 will
be useful to predict the presence of the long allele of \textit{Lcol-A1} in diverse germplasm.

\textbf{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 \textit{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 \textit{Lcol-A1} more efficiently in future cultivars.

\textbf{Methods}

\textbf{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.

\textbf{Bulked-segregant analysis}

One-hundred and eighty-eight (188) BC$_1$F$_2$ 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 BC$_1$F$_2$ 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.

\textbf{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 BC$_1$F$_2$ plants were screened with a marker derived from the \textit{Rht-B1b} dwarfing allele [13] to identify 54 BC$_1$F$_2$ plants that lacked \textit{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 \textit{IWA164} which was mapped in the 9 K SNP consensus map of wheat [10] to the 1A region identified in BSA, and BC$_3$F$_3$ progeny were phenotyped for coleoptile length under controlled conditions (see above). Similarly, the plants were genotyped with markers \textit{IWA3374} and \textit{IWA5435} from the 9 K SNP consensus map, which flanked the 1A region identified in BSA. The BC$_1$ 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, BC$_2$F$_3$ progeny from BC$_2$F$_2$ plants that were heterozygous for markers in the 1A region were screened withflanking markers \textit{IWA3374} and \textit{IWA5435}. DNA was extracted from half-seeds of 900 BC$_2$F$_3$ 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.

\textbf{Validation of the 1A region}

The 1A region was transferred from HI25M to Espada (an Australian cultivar) through backcrossing. BC$_3$F$_3$ plants were genotyped for \textit{Rht-D1b} [13], \textit{Rht18} [21], and the 1A locus. Lines that were homozygous wild-type for \textit{Rht-D1}, homozygous dwarf for \textit{Rht18}, and heterozygous for the 1A locus were selfed to produce BC$_3$F$_3$ 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.

\textbf{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 BC$_1$F$_3$ 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 IWBS8229 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 isoline 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.

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**Authors’ contributions**

Wrote the manuscript, developed germplasm and performed experiments: GP and TP. Undertook 9 K SNP BSA analysis and reviewed the manuscript: BJB and GJR. Performed experiments: JH. 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: MJJ. 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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