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Identification and Validation of a Novel Major QTL Controlling Leaf Pubescence in the Chinese Wheat Landrace 'Baimaomai'

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Abstract: Leaf pubescence is an important trait closely associated with plant adaptability to specialized habitats. Baimaomai (BMM) is a wheat (*Triticum aestivum* L.) landrace originating from the high-altitude, drought-prone environment of Sichuan Province, China with long, dense leaf pubescence. A population of 234 recombinant inbred lines (F_{10}) developed from the cross between Chuanmai104 (CM104), which lacks leaf pubescence, and BMM with pubescent leaves, was used to conduct a phenotypic evaluation of leaf pubescence. Three quantitative trait loci (QTLs) were detected on chromosome arms 7BS, 3DL and 3AL using a high-density wheat 50K single-nucleotide polymorphism array in four environments. The QTLs were designated *QLp.saas-7BS*, *QLp.saas-3DL* and *QLp.saas-3AL*. *QLp.saas-3AL*, derived from BMM, and *QLp.saas-3DL*, derived from CM104, were new minor-effect loci. *QLp.saas-7BS*, derived from BMM, was a novel major-effect locus detected in all environments and was localized in a 0.48 Mb interval on chromosome arm 7BS based on the wheat 'Chinese Spring' reference genome. *QLp.saas-7BS* explained up to 40.77% of the total phenotypic variance. KASP markers tightly linked to *QLp.saas-7BS* were developed and verified. The present results provide valuable information for further fine mapping, cloning, and marker-assisted selection with *QLp.saas-7BS* in wheat.

Keywords: wheat; landrace; Baimaomai; leaf pubescence; QTL

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1. Introduction

Leaf pubescence is an adaptive morphological trait and a common characteristic of angiosperms [1,2]. Leaf pubescence plays an important role in plant growth, development, and environmental adaptation. Pubescence refers to the presence of trichomes, which are ubiquitous in plants and comprise uni- or multi-cellular outgrowths from the epidermis [3,4]. Some trichomes perform a secretory function and may secrete a variety of chemicals, such as terpenoids and phenylpropanoids, which are important for plant–animal [5] or plant–fungal interactions [6].

With regard to resistance to biotic stresses, leaf pubescence may improve plant resistance to insects by affecting the preferences and behavior of diverse insects [7], such as cereal leaf beetle [8,9], yellow sugarcane aphid and greenbug [10], and bird cherry-oat aphid in wheat [11]. The degree and density of leaf pubescence is associated with variation in the degree of resistance or susceptibility to various insect pests [12–14]. In addition, pubescence may serve as a barrier to infection by foliar pathogens [15]. The presence of

trichomes may reduce the relative humidity at the leaf surface, which is unfavorable for germination of fungal spores [15]. Some trichome-related plant proteins are reported to play an essential role in resistance to foliar pathogens, such as TRICHOME BIREFRINGENCE (TBR)-like proteins in rice [16] and α-1,3-glucanase in Arabidopsis thaliana [17].

In addition, plant leaf pubescence is important in the response to abiotic stresses. Many studies have observed the important protective value of leaf pubescence under drought [18,19], thermal stress [2,20,21], and intense solar radiation [20,21]. Drought also leads to increased pubescence production in plants as an adaptative response [3]. Many plants growing in semi-arid environments maintain water contents by foliar absorption of water aided by pubescence [6]. For example, pubescence reduces evaporation and regulates plant tissue temperature by increasing the thickness of the epidermis and the content of long-chain fatty acids [22]. Pubescence also plays an important role in moisture absorption from dew which decreases the water potential of drought-stressed leaves, and contributes to plant photosynthetic performance under water stress [23–25]. The density of leaf pubescence is an important component of adaptation for drought tolerance as revealed by genetic analysis of leaf pubescence density and water status traits in soybean [26]. Notably, leaf pubescence plays an important role in plant development and productivity because it influences photosynthesis and therefore transpiration, respiration, and grain-yield formation [7,23,27,28].

In wheat, pubescence traits are expressed in various tissues and are regulated by different genes. For instance, glume pubescence is controlled by the gene *Hg*, also named *Hg1* (weakly hairy phenotype), located on chromosome arm 1AS [29,30]. The gene *Hg2* (very hairy phenotype) was localized to chromosome 2BL of synthetic hexaploid wheat using a bulked segregant analysis and RNA-sequencing approach in a $F_{2:3}$ population. Luo et al. [31] detected 37 differentially expressed genes and 39 single-nucleotide polymorphisms (SNPs) in the *Hg* gene region on chromosome 1AS by transcriptome analysis. A hairy leaf sheath in bread wheat introgressed from *Aegilops tauschii* Coss. is mainly controlled by the quantitative trait locus (QTL) *QLsh.saas-4D*, which is significantly positively associated with yield and yield-related traits, including grain yield, grain weight, spike number per square meter, and grain weight per spike [32]. Hairiness of the leaf margins and auricles in wheat are regulated by the QTLs *QHl.ipk-4B* and *QHl.ipk-4D* on chromosome 4BL, and *QPa.ipk-4B* and *QPa.ipk-4D* on chromosome 4DL, respectively [33].

Numerous genes that regulate leaf pubescence in wheat have been identified. The dominant gene *Hl1* is localized on chromosome 4BL [33,34]. A second dominant gene, *Hl2*, was localized on chromosome 7BS in the pubescent Chinese cultivar 'Hong-Mang-Mai' grown on the arid Loess Plateau [35]. A third pubescence-related gene, *Hl3*, was detected by genetic analysis but its precise genomic position remains unknown [36]. *Hl1* and *Hl3* more strongly affect initiation and development of leaf pubescence, whereas *Hl2* affects leaf trichome length [36]. A major gene, *Hl2aesp*, on chromosome 7BS, regulates the leaf hair length, which was introgressed from *Ae. speltoides* Tausch into common wheat [37]. *Hl2aesp* is considered to be an allele of *Hl2* [33]. In addition, a gene on chromosome 7D of the cultivar 'Novosibirskaya 67' increases pubescence density [38]. More recently, Simonov et al. [39] used an introgression line obtained by crossing 'S29' with tetraploid Triticum timopheevii (Zhuk.) Zhuk. to identify and map the novel dominant gene *Hltt* to the distal region of chromosome 5AL, which controls the formation of long trichomes. The *Hltt* gene exerts a suppressive effect both on dominant and recessive alleles of *Hl1* and *Hl3* in different genetic backgrounds. In contrast, *Hl1* and *Hl3* may enhance the effect of *Hltt* in specific genetic backgrounds, which results in formation of an abundance of extremely long trichomes [39].

In the present study, we used Baimaomai (BMM), a hexaploid wheat landrace originating from a specialized environment characterized by high altitude, intense radiation, drought, and aridity in the western plateau region of Sichuan, China. BMM exhibits a number of specific physiological traits, including long and dense pubescence on the leaves. The aim of the study was to map QTLs for leaf pubescence of BMM using a genetically

isolated population derived from crosses between Chuanmai104 (CM104; non-pubescent leaves) and BMM, and high-throughput SNP microarray technology. Linkage markers linked to the major QTLs were developed and verified. The results will contribute to an improved understanding of the genetic control of leaf pubescence in wheat.

2. Materials and Methods

2.1. Plant Materials

Baimaomai, a hexaploid wheat landrace with long, dense pubescence on the leaves, was originally collected from the western plateau region of Sichuan, China. Chuanmai104, an elite commercial wheat cultivar that lacks leaf pubescence, was developed by the Crop Research Institute, Sichuan Academy of Agricultural Sciences (CRI-SAAS) and released in 2012 [40]. The leaf pubescence phenotypes of BMM and CM104 are shown in Figure 1. A recombinant inbred line (RIL) mapping population comprising 234 F_{10} lines was developed from the cross between CM104 and BMM, and was used to map QTLs associated with leaf pubescence. The RIL population is hereafter referred to as CB-RILs. In addition, 110 F_9 RILs were developed from the cross between 14Pin16 (a hexaploid wheat with non-pubescent leaves) and BMM, hereafter abbreviated as PB-RILs which were intended for marker validation. A total of 62 lines, comprising CM104, 14Pin16, BMM, 48 CB-RILs, and 11 PB-RILs, were used to validate the developed kompetitive allele-specific PCR (KASP) markers closely linked to the major QTL *QLp.saas-7BS* (Supplementary Table S1). All materials were provided by CRI-SAAS.

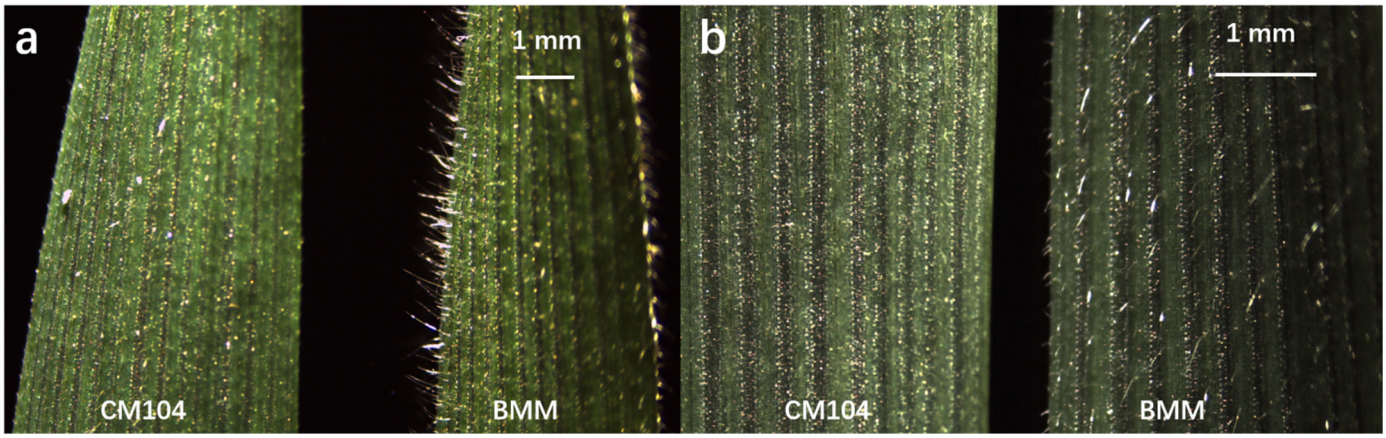

Figure 1. Leaf pubescence phenotypes of the parents Chuanmai104 (CM104) and Baimaomai (BMM) observed with a stereomicroscope at the heading stage: (**a**) the lateral observation of leaf surface; (**b**) the frontal observation of leaf surface.

2.2. Phenotypic Evaluation

The RILs and parents were planted in four environments comprising Pixian (30°81′ N, 103°88′ E) in 2019–2020 (2020PX) and 2020–2021 (2021PX), and Guanghan (30°99′ N, 104°25′ E) in 2019–2020 (2020GH) and 2020–2021 (2021GH) in Sichuan Province of China. The RILs and parents in all environments were sown in random blocks. Each line was planted in a single 1 m long row, and with 25 cm inter-row spacing between RILs. The sowing density was 10 seeds per row with 10 cm spacing between plants in these specific areas. Field management followed conventional practices used in wheat production. Five representative flag leaves per line were selected for phenotypic trait measurement at the heading stage. The trichome length and density of leaf pubescence were observed in an area of 1 cm^2 in the center of the flag leaves using a Leica EZ4 HD stereo microscope (Leica Microsystems company, Wetzlar, Germany; Figure 1). The phenotypic traits were scored using a scale from 0 to 3, with 0 representing no pubescence, 1 for short and sparse pubescence, 2 for pubescence of moderate length and density, and 3 for the longest and most dense pubescence. The statistics of phenotypic data were conducted using Microsoft

Excel, and the correlation analysis among the different environments were conducted using the software IBM SPSS Statistics 25.0 (International business machines corporation, Armonk, NY, USA).

2.3. QTL Mapping and Candidate Genes Prediction

The genetic linkage map used in this study was constructed using 3800 polymorphic SNP markers from the wheat 50K array developed by CapitalBio Corporation (Beijing, China) and synthesized by Affymetrix [40]. The QTL analysis was conducted by inclusive composite interval mapping using QTL IciMapping 4.1 software [40]. The QTLs were mapped at a logarithm of odds threshold of 2.5 based on 1000 permutations and a walk speed of 1.0 cM, with $p = 0.001$, by stepwise regression. The QTL effect was estimated as the proportion of the total phenotypic variance explained by the QTL.

Based on the flanking sequences, the physical distance between the marker interval of each QTL was determined using the Chinese Spring IWGSC RefSeq v2.1 genome assembly accessed on the International Wheat Genome Sequencing Consortium (IWGSC) website (http://www.wheatgenome.org/) [41]. According to the physical location of the flanking sequences, the candidate genes for the major QTL *QLp.saas-7BS* were predicted with reference to IWGSC RefSeq v2.1, the wild emmer reference genome (Zavitan WEWSeq v2.0), and durum wheat reference genome (Svevo RefSeq Rel. 1.0) accessible in the Triticeae Multi-omics Center database (http://202.194.139.32/).

2.4. Marker Development and QTL Validation

Based on the QTL mapping results, we converted the flanking SNP markers AX-86172908 and AX-86175290 tightly linked to the major QTL *QLp.saas-7BS* into KASP markers, designated KASP-AX-86172908 and KASP-AX-86175290, respectively. Forty-four lines randomly selected from the RIL population and BMM were used to perform genotyping using two sets of KASP markers, respectively. The reaction mixture for genotype detection comprised 0.75 μL DNA, 2.85 μL deionized water, 5 μL KASP Master Mix (LGC Biosearch Technologies, Hoddesdon, UK), and 1.4 μL primers. Reactions were conducted using the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The primers for KASP-AX-86172908 (FAM: GAAGGTGACCAAGTTCATGCTTGCACAACCAGTCAGCAGAAA; HEX: GAAGGTCGGAGTCAACGGATTTGCACAACCAGTCAGCAGAAG; Reverse: TCGGGATATTGAATTCTTGAGCTAC) and KASP-AX-86175290 (FAM: GAAGGTGACCAAGTTCATGCTTGCAGTGAAGTTATTAACACC; HEX: GAAGGTCGGAGTCAACGGATTTGCAGTGAAGTTATTAACACT; Reverse: ATATGCAGTACAGATCTTTCACAG) were designed according to the sequence of AX-86172908 and AX-86175290, respectively. The lines were divided into two categories based on the genotype of the two sets of KASP markers: the first category comprised lines with homozygous alleles from BMM, and the second category comprised lines with homozygous alleles from CM104. The significance of differences between the two categories ($p < 0.05$) was statistically analyzed using IBM SPSS Statistics 25 software.

3. Results

3.1. Phenotypic Evaluation

The leaf pubescence of CM104 (with non-pubescent leaves) was scored as 0 and BMM (with the longest and highest-density leaf pubescence) was scored as 3 (Figure 1, Table 1). Thus, the length and density of leaf pubescence differed significantly between CM104 and BMM. The 234 RILs differed in pubescence phenotypes ranging in scale from 0 to 3 in the four environments at the heading stage (Table 1). The phenotype of each line was consistent across all environments. Correlation coefficients among the different environments were significant ($p < 0.01$) and ranged from 0.4815 to 0.9139.

Table 1. LP density of the parents and recombinant inbred lines at the heading stage.

Environments	LP Density of Parents		LP Density of RIL Population							
	CM104	BMM	Mean	Variance	SD	Skewness	Kurtosis	Min	Max	H^2
2020PX	0	3	1.03	1.03	1.01	0.57	−0.86	0	3	63.18
2020GH	0	3	1.17	1.12	1.06	0.42	−1.07	0	3	65.39
2021PX	0	3	1.56	1.07	1.03	−0.06	−1.15	0	3	58.47
2021GH	0	3	1.67	0.98	0.99	−0.12	−1.05	0	3	51.39

LP, leaf pubescence; SD, standard deviation; H2, broad-sense heritability; CM104, Chuanmai104; BMM, Baimaomai; 2020PX, 2021PX, 2020GH, and 2021GH represent the four environments: Pixian in 2020, Pixian in 2021, Guanghan in 2020, and Guanghan 2021, respectively.

3.2. QTL Mapping for Leaf Pubescence

Three putative QTLs associated with leaf pubescence were detected and localized on chromosomes 7BS, 3AL, and 3DL, and were designated *QLp.saas-7BS*, *QLp.saas-3AL*, and *QLp.saas-3DL*, respectively (Figure 2, Table 2). *QLp.saas-7BS* and *QLp.saas-3AL* were derived from BMM, whereas *QLp.saas-3DL* was derived from CM104.

Table 2. QTLs for leaf pubescence by inclusive composite interval mapping in the recombinant inbred lines population of Chuanmai104 × Baimaomai in four environments. The physical position of markers is in relation to the Chinese Spring reference genome (IWGSC RefSeq v2.1).

QTL.	ENV	CHR	Left Marker (Position/bp)	Right Marker (Position/bp)	Physical Interval (Mb)	LOD	PVE (%)	ADD	Source
QLp.saas-7BS	2020PX	7BS	AX-86175290 (61106266)	AX-86172908 (60629908)	0.48	38.51	40.77	−0.64	BMM
	2020GH	7BS	AX-86175290 (61106266)	AX-86172908 60629908)	0.48	13.22	14.16	−0.34	BMM
	2021PX	7BS	AX-86175290 (61106266)	AX-86172908 (60629908)	0.48	29.97	31.28	−0.58	BMM
	2021GH	7BS	AX-86175290 (61106266)	AX-86172908 (60629908)	0.48	26.11	26.22	−0.55	BMM
QLp.saas-3DL	2020PX	3DL	AX-111450459 (531264108)	AX-110717155 (520933666)	10.33	8.16	6.46	0.26	CM104
	2020GH	3DL	AX-95258719 (540919250)	AX-95225989 (548702914)	7.78	7.05	7.31	0.24	CM104
	2021PX	3DL	AX-89725997 (536000818)	AX-95258719 (540919250)	4.92	5.32	4.50	0.22	CM104
	2021GH	3DL	AX-89725997 (536000818)	AX-95258719 (540919250)	4.92	4.21	3.64	0.21	CM104
QLp.saas-3AL	2020PX	3AL	AX-110027317 (649937635)	AX-111628960 (653099586)	3.16	5.03	3.77	−0.20	BMM
	2020GH	3AL	AX-94511878 (663632445)	AX-110378150 (653031240)	10.60	4.43	4.44	−0.19	BMM
	2021PX	3AL	AX-109410907 (667677536)	AX-111122851 (664279684)	3.40	4.04	3.23	−0.19	BMM
	2021GH	3AL	AX-111696842 (673592990)	AX-109410907 (667677536)	5.92	3.60	3.07	−0.19	BMM

ENV, environment; CHR, chromosome; LOD, logarithm of odds; PVE, phenotypic variance explained; ADD, additive effect; BMM, Baimaomai; CM104, Chuanmai104; 2020PX, 2021PX, 2020GH, and 2021GH represent the four environments: Pixian in 2020, Pixian in 2021, Guanghan in 2020, and Guanghan 2021, respectively.

As a stable major locus, *QLp.saas-7BS* was detected in all four environments and explained 14.16–40.77% of the total phenotypic variance (Table 2). Based on the Chinese Spring IWGSC RefSeq v2.1 reference genome [41], the major allele of *QLp.saas-7BS* was localized in a 0.48 Mb genomic region between the markers AX-86175290 and AX-86172908 on the short arm of chromosome 7B; the genetic distance between the markers was 0.8 cM. On the basis of the physical position of the flanking sequences of the SNP markers, 10 candidate genes were located in this interval of the Chinese Spring genome.

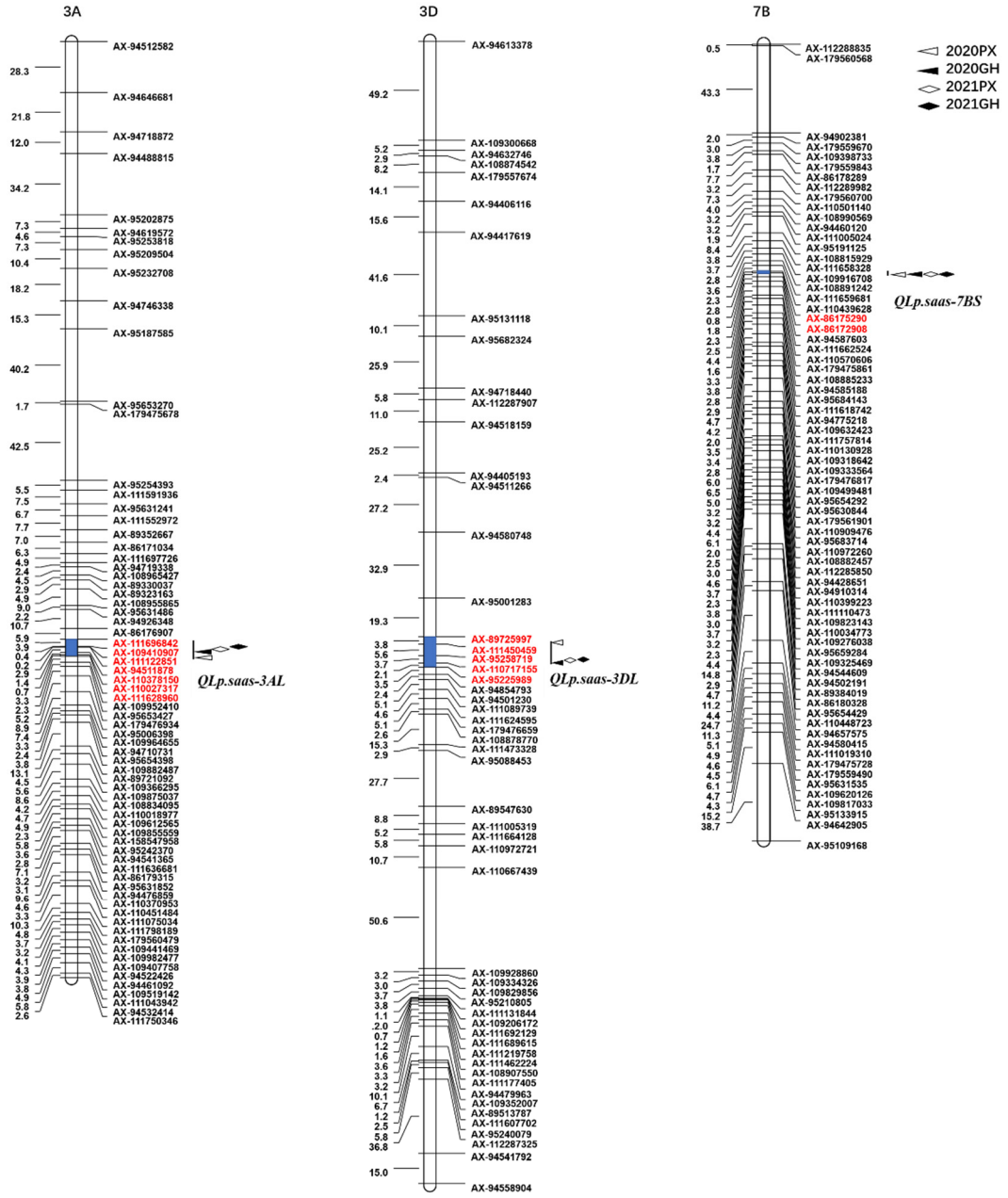

Figure 2. Mapping of QTLs associated with leaf pubescence in the CM104 × BMM recombinant inbred lines population grown in four environments; 2020PX, 2021PX, 2020GH, and 2021GH represent the four environments: Pixian in 2020, Pixian in 2021, Guanghan in 2020, and Guanghan 2021, respectively.

With reference to the genome sequence of durum wheat (Svevo RefSeq Rel. 1.0), *QLp.saas-7BS* was localized in a 0.48 Mb interval between the physical locations 58.65 Mb and 59.13 Mb on chromosome 7BS. Five candidate genes were located in this interval. Furthermore, *QLp.saas-7BS* was localized to a 0.46 Mb interval between the physical positions 66.97 Mb and 67.43 Mb on chromosome 7BS of the wild emmer reference genome (Zavitan WEWSeq v2.0). Five candidate genes were also located in this interval (Figure 3).

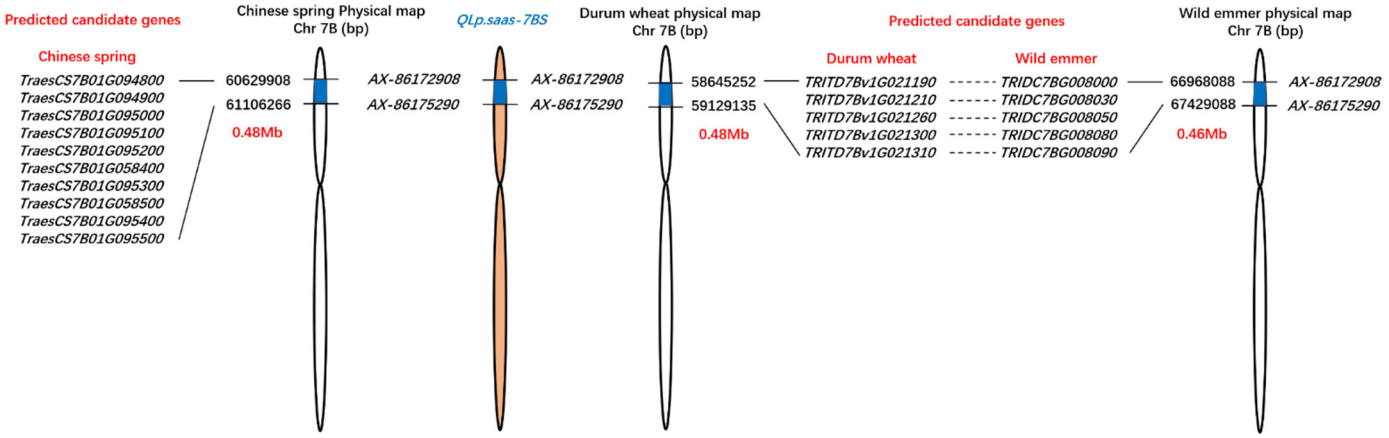

Figure 3. Physical map and candidate genes of the major-effect QTL *QLp.saas-7BS* in relation to the Chinese Spring, wild emmer, and durum wheat reference genome sequences accessed in the Triticeae Multi-omics Center database (http://202.194.139.32/).

A minor-effect locus, *QLp.saas-3AL*, derived from BMM was identified in all environments and was localized on the long arm of chromosome 3A. This QTL explained 3.07–4.44% of the total phenotypic variance. The minimum physical distance of *QLp.saas-3AL* was mapped to a 3.16 Mb region between AX-110027317 and AX-111628960 in 2020PX, and the maximum physical distance was localized to a 10.60 Mb region between AX-94511878 and AX-110378150 in 2020GH. In addition, *QLp.saas-3AL* was detected in 2021PX and 2021GH, and the physical interval was 3.4 Mb between AX-109410907 and AX-111122851, and 5.92 Mb between AX-111696842 and AX-109410907, respectively (Table 2, Figure 2).

An additional minor locus, *QLp.saas-3DL*, derived from CM104, was detected in all environments and mapped on the long arm of chromosome 3D, and explained 3.64–6.46% of the total phenotypic variance. The physical interval of *QLp.saas-3DL* was localized to a 4.92 Mb genome region between AX-89725997 and AX-95258719 in 2021PX and 2021GH. However, in 2020PX, the physical interval of *QLp.saas-3DL* was localized to the 10.33 Mb genome region between AX-111450459 and AX-110717155 in 2020PX, and to the 7.78 Mb region between AX-95258719 and AX-95225989 in 2020GH (Table 2, Figure 2).

3.3. Validation of the Novel Major QTL QLp.saas-7BS

A total of 62 lines randomly selected from among the CB-RIL and PB-RIL populations were used to evaluate the effects of *QLp.saas-7BS*. The newly developed markers KASP-AX-86172908 and KASP-AX-86175290 tightly linked to *QLp.saas-7BS* were used to evaluate the alleles derived from BMM. Based on the presence or absence of the allele from BMM, all 62 tested lines showed polymorphism by detection with the two markers and were classified into two categories (Figure 4, Table S1). The difference between the two categories was significant ($p < 0.05$) (Figure 4).

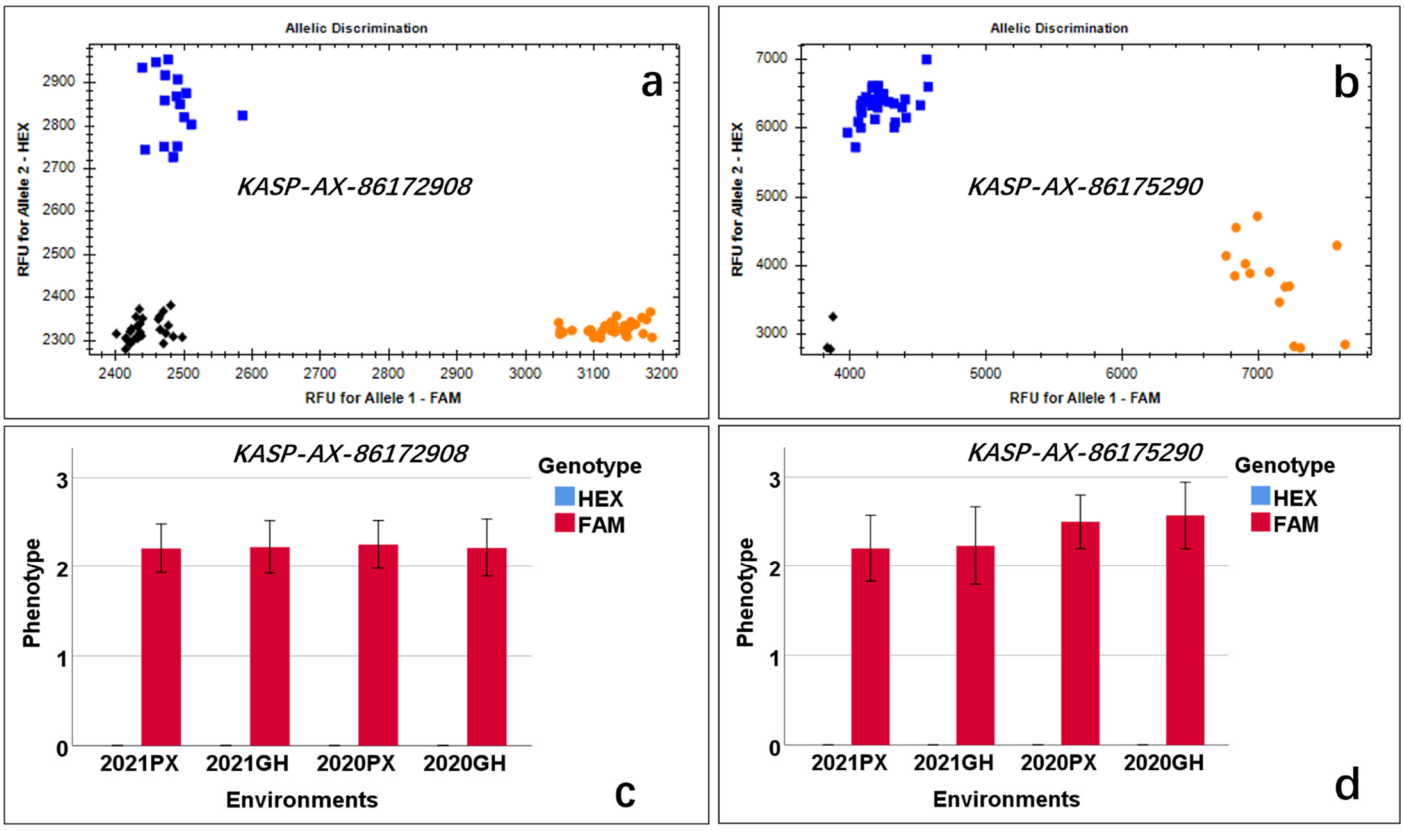

Figure 4. Allelic discrimination of the markers KASP-AX-86172908 and KASP-AX-86175290. Blue squares represent the lines lacking leaf pubescence (HEX fluorescence), orange circles represent the lines with leaf pubescence (FAM fluorescence), and black diamonds represent the negative control. (**a**) Allelic discrimination of KASP-AX-86172908 included 16 lines without leaf pubescence (blue squares) and 28 lines with leaf pubescence (orange circles). (**b**) Allelic discrimination of KASP-AX-86175290 included 30 lines without leaf pubescence (blue squares) and 14 lines with leaf pubescence (orange circles). (**c**,**d**) Effects of *QLp.saas-7BS* in validation lines detected by KASP-AX-86172908 and KASP-AX-86175290, respectively; the *y*-axis is the phenotype score, and the *x*-axis is the four environments; the phenotype score of lines without pubescence was 0, which is represented by a blue bar (HEX); the phenotype score of other lines is represented by a red bar (FAM fluorescence).

4. Discussion

4.1. QLp.saas-7BS Is a Novel Major QTL

The dominant gene *Hl2* was previously localized on chromosome 7B in the pubescent Chinese wheat cultivar 'Hong-Mang-Mai' by monosomic and phenotypic analysis of F_1 plants and the F_2 population developed from the cross between 'Hong-Mang-Mai' and 'Chinese Spring'. The *Hl2* gene was subsequently mapped to the short arm of chromosome 7B by telosomic and phenotypic analysis of BC_1F_1 populations developed from the backcross between monotelodisomic F_1 hybrids and 'Chinese Spring' [35]. However, the precise physical position of *Hl2* remains unclear. Pshenichnikova et al. [37] identified a major gene, *Hl2aesp*, on chromosome 7BS that controls the length of leaf trichomes using the same method. The gene *Hl2aesp* was introgressed from *Ae. speltoides* into common wheat. Based on an allelism test of F_2 individuals from the cross between 'Hong-Mang-Mai' carrying *Hl2* and the wheat/*Ae. speltoides* introgression line 102/00I carrying *Hl2aesp*, *Hl2aesp* was concluded to be allelic to *Hl2* and was localized to the interval between Xgwm255 and Xgwm400 [33,36]. On the basis of marker sequences and the Chinese Spring IWGSC RefSeq v2.1 genome assembly [41], *Hl2aesp* was approximately localized to a more distal position within the 26.8–34.3 Mb interval on the short arm of chromosome 7B. In addition to chromosome 7B, QTLs associated with leaf pubescence have been identified on chromosomes 7A [42] and 7D [38]. Therefore, it is speculated that a series of homoeologous genes regulating leaf pubescence might be present on homoeologous group 7 chromosomes [38].

In the present study, *QLp.saas-7BS*, a major QTL derived from BMM, was localized to chromosome 7BS in a 0.48 Mb interval between the markers AX-86175290 (60.63 Mb) and AX-86172908 (61.11 Mb) in the Chinese Spring IWGSC RefSeq v2.1 genome [41]. The results showed that *QLp.saas-7BS* was closer to the centromere than *Hl2aesp*, which was closer to the distal end of 7BS. Therefore, it is speculated that *QLp.saas-7BS* might be a novel major QTL and distinct from *Hl2aesp*, although the relationship among these genes requires further study. Effective KASP markers tightly linked to *QLp.saas-7BS* were developed and verified, which would be helpful to conduct marker-assisted selection for this morphological trait in the future.

4.2. Candidate Genes Located in the Interval for QLp.saas-7BS

Based on the Chinese Spring IWGSC RefSeq v2.1 reference genome, the physical interval of *QLp.saas-7BS* contained 10 candidate genes (Figure 3). Four of these genes, namely *TraesCS7B01G094800*, *TraesCS7B01G094900*, *TraesCS7B01G095000*, and *TraesCS7B01G058400*, were annotated to encode a glutamate receptor, which is associated with various physiological and developmental processes [43]. It is speculated that the development of leaf pubescence is regulated by glutamate receptor signaling. In addition, a plant glutamate receptor may contribute to the ability to respond to an attack from a pest or pathogen [44], and enhances the heat tolerance of maize seedlings [45]. Interestingly, one candidate gene, *TraesCS7B01G058500*, was annotated to encode a mannitol transporter, which contributes to the plant response to drought stress [46,47]. A mannitol transporter is also associated with resistance to biotic and abiotic stresses [48]. *TraesCS7B01G095500* was annotated to encode a pentatricopeptide repeat (PPR) superfamily protein, which are mainly localized in chloroplasts and mitochondria and are reported to be involved in the regulation of plant growth and development under stresses [40]. Among the other candidate genes, *TraesCS7B01G095400* was annotated to encode a DNA polymerase, which is responsible for DNA synthesis during the process of DNA replication and the transfer genetic information between generations. Three candidate genes, namely *TraesCS7B01G095100*, *TraesCS7B01G095200*, and *TraesCS7B01G095300*, were annotated to encode a retrotransposon protein, which represents a complex fraction of repetitive DNA present in most eukaryotes [49] and is involved in generation of mutations through insertions near or within genes [50]. These candidate genes located in the physical interval of *QLp.saas-7BS* will be evaluated and validated for the association to the leaf pubescence in further studies.

Furthermore, *QLp.saas-7BS* was localized to a narrow physical interval on chromosome 7BS of durum wheat (0.48 Mb) and wild emmer (0.46 Mb). The reference genomes for durum wheat (Svevo RefSeq Rel. 1.0) and wild emmer (Zavitan WEWSeq v2.0) each contained five candidate genes in the target interval (Figure 3). These genes will contribute to fine mapping and cloning of the novel major QTL *QLp.saas-7BS* in the future.

4.3. Other QTLs

Taketa et al. [35] detected a highly significant segregation ratio for a critical monosomic combination from a monosomic analysis of F_2 plants derived from the cross between Chinese Spring 3D monosomics and Hong-Mang-Mai, but the monosomic analysis was contrary to the results of phenotypic analysis. Therefore, it was considered that chromosome 3D did not carry a gene for leaf pubescence [35]. Interestingly, a stable and minor QTL, *QLp.saas-3DL*, derived from CM104, was also identified on chromosome 3DL in all four environments in the present study (Table 2). However, the leaf blade of CM104 is non-pubescent (Figure 1), but has been reported to show leaf sheath hairiness when introgressed from *Ae. tauschii* [32]. The possibility of a relationship between leaf sheath hairiness and *QLp.saas-3DL* requires further investigation in the future. Furthermore, the relationship between chromosome 3D and leaf pubescence remains uncertain and requires further study.

To date, genes controlling leaf pubescence on chromosome 3A have not been reported previously in wheat. *QLp.saas-3AL*, as a stable and minor QTL, was first reported to be

localized on chromosome 3AL derived from BMM (Table 2). In barley (*Hordeum vulgare* L.), the gene *Pub* on chromosome 3HL is responsible for leaf pubescence [36]. The collinearity between *QLp.saas-3AL* and *Pub* would be crucial warranting further research to understand the functions of both loci.

Leaf pubescence, as an important adaptive trait, reflects resistance to biotic and abiotic stresses, such as insect and pathogen attack, drought, and radiation, and can also enhance photosynthetic efficiency. In the present study, three gene loci associated with leaf pubescence were identified in wheat, comprising the major QTL *QLp.saas-7BS* and two minor QTLs *QLp.saas-3AL* and *QLp.saas-3DL*. The present results will contribute to a better understanding of the formation and development of leaf pubescence in wheat, and the newly developed markers will be useful to detect the *QLp.saas-7BS* related to leaf pubescence in the future.

5. Conclusions

Leaf pubescence is an important phenotypic trait closely associated with plant adaptability. Three QTLs related to leaf pubescence were detected in this study, namely *QLp.saas-3AL*, *QLp.saas-3DL*, and *QLp.saas-7BS*. The QTLs *QLp.saas-3AL* and *QLp.saas-3DL* are minor-effect loci. *QLp.saas-7BS* derived from BMM is a novel major-effect locus that was detected in all four environments and mapped to a 0.48 Mb interval on chromosome arm 7BS, and explained up to 40.77% of the total phenotypic variance. We also developed and verified KASP markers tightly linked to *QLp.saas-7BS*. These results will be helpful for marker-assisted selection, fine mapping, and cloning of genes associated with leaf pubescence in wheat.

6. Patents

A patent application has been submitted in China for the primer sequences of all the KASP markers in this study.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112237/s1, Table S1: The total of 62 lines were used to validate the developed KASP markers including KASP-AX-86172908 and KASP-AX-86175290.

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