agronomy

MDPI

Article

Quantitative Trait Locus Analysis for Panicle and Flag Leaf Traits in Barley (*Hordeum vulgare* L.) Based on a High-Density Genetic Linkage Map

Yichen Ye, Shuting Wen, Guo Zhang, Xingzhe Yang, Dawei Xue, Yunxia Fang and Xiaoqin Zhang *

College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China; 2022111010031@stu.hznu.edu.cn (Y.Y.); 2022111010026@stu.hznu.edu.cn (S.W.); zhangguo@stu.hznu.edu.cn (G.Z.); 2023111010016@stu.hznu.edu.cn (X.Y.); dwxue@hznu.edu.cn (D.X.); yxfang12@hznu.edu.cn (Y.F.)
* Correspondence: zxq@hznu.edu.cn

Abstract: The yield of barley (*Hordeum vulgare* L.) is determined by many factors, which have always been research hotspots for agronomists and molecular scientists. In this study, five important agronomic traits related to panicle and flag leaf, including awn length (AL), panicle length (PL), panicle neck length (NL), flag leaf length (LL) and flag leaf width (LW), were investigated and quantitative trait locus (QTL) analyses were carried out. Using a high-density genetic map of 134 recombinant inbred lines based on specific-locus amplified fragment sequencing (SLAF-seq) technology, a total of 32 QTLs were identified, which explained 12.4% to 50% of the phenotypic variation. Among them, *qAL5*, *qNL2*, *qNL3*, *qNL6*, *qPL2*, and *qLW2* were detected in 3 consecutive years and all of the contribution rates were more than 13.8%, revealing that these QTLs were stable major QTLs and were less affected by environmental factors. Furthermore, LL and LW exhibited significant positive correlations and the localization intervals of *qLL2* and *qLL3* were highly overlapped with those of *qLW2* and *qLW3*, respectively, indicating that *qLL2* and *qLW2*, *qLL3* and *qLW3* may be regulated by the same genes.

Keywords: barley; recombinant inbred line; panicle; flag leaf; QTL

Citation: Ye, Y.; Wen, S.; Zhang, G.; Yang, X.; Xue, D.; Fang, Y.; Zhang, X. Quantitative Trait Locus Analysis for Panicle and Flag Leaf Traits in Barley (*Hordeum vulgare* L.) Based on a High-Density Genetic Linkage Map. *Agronomy* **2024**, *14*, 2953. https://doi.org/10.3390/agronomy14122953

Academic Editor: Matthew Hegarty

Received: 25 October 2024
Revised: 1 December 2024
Accepted: 9 December 2024
Published: 11 December 2024

1. Introduction

In recent years, there has been a notable increase in the global demand for food, which has led to an intensified focus on enhancing crop yields. Barley (*Hordeum vulgare* L.), one of the earliest domesticated food crops, has been widely cultivated worldwide due to its high yield potential and important roles in various industries, including animal feeding, brewing and food production [1]. Improving the yield by systematically optimizing agronomic traits to better meet the growing food demand in barley breeding has become a consensus [2]. Therefore, the quantitative trait locus (QTL) analysis of barley yield-related traits can not only understand the complex genetic mechanism, but also lay the foundation for molecular breeding.

Barley yield is a complex quantitative trait, which is directly or indirectly affected by other traits. Among them, the panicle shape and grain size directly determine crop yield [3], while flag leaf length and width also affect yield by influencing the photosynthesis rate [4,5]. Although the awn is not an important organ for the growth and reproduction of triticeae crops, it can act as a photosynthetic organ and is one of the potential pathways to increase yield by significantly increasing photosynthetic products [6]. In addition, the awn also plays a role in resisting insect pests and bird pecking, which indirectly affect yield [7]. It is reported that the length of the panicle neck is also an important agronomic trait in barley breeding. A longer panicle neck increases the light and air exchange of organs above the flag leaf, reduces the incidence of fusarium head blight in the panicle and thus increases grain filling and thousand-grain weight [8]. However, too long a panicle

neck length will lead to the excessive elongation of plant height, resulting in lodging [9]. Therefore, the appropriate awn length and panicle neck length are beneficial to the increase in plant yield [10].

Using a double haploid (DH) population, Wang et al. [11] constructed a high-density genetic map of barley and performed QTL analysis for 10 traits such as main panicle length, grain number per panicle and thousand-grain weight. A total of three main panicle length QTLs were detected on chromosomes 2H and 7H, explaining the contribution rates of 1.93 to 52.72. The genome wide association (GWA) analysis of yield-related traits was also carried out by using barley germplasm resources consisting of 185 cultivated and 38 wild genotypes, and seven and one QTLs related to panicle length were identified on chromosomes 2H, 3H, 4H and 5H in wet and dry environments [12]. Four flag leaf traits of barley (flag leaf thickness, length, width and area) were investigated, and five QTLs associated with flag leaf length were localized on chromosomes 1H, 2H, 3H, 4H and 6H, and two QTLs associated with flag leaf width were localized on chromosome 2H, explaining 5.8–17.9% of the phenotypic variation [13]. Du et al. [14] detected the QTLs for the length and area of flag, and the second, third and fourth leaves in barley. A total of 57 QTLs were identified, of which 6 relating to flag leaf length were located on chromosomes 2H and 7H, with variances ranging from 5.30 to 37.11%. Moreover, 12 QTLs for awn length distributed on all the 7 barley chromosomes were detected by a multiparent mapping population, in which the QTL located on chromosome 7HL explained the highest contribution rate [15]. These results provide a genetic basis for the study of panicle and flag leaf traits in barley.

Although barley has always been an important food crop, its large genome renders it difficult to construct high-density genetic maps using conventional methods [16]. Therefore, it is more feasible to construct genetic maps by SLAF-seq technology [17]. In this study, the 134 recombinant inbred lines (RILs) derived from the cross between Golden Promise (GP) and wild variety H602 were used to determine five agronomic traits, including panicle length (PL), awn length (AL), panicle neck length (NL), flag leaf length (LL) and flag leaf width (LW). The QTL analysis was conducted by a high-density genetic map based on SLAF markers, and a total of 32 QTLs were detected in consecutive three years. In this study, a scientific basis is provided for the genetic improvement of panicle and flag leaf traits in barley.

2. Materials and Methods

2.1. Plant Materials

A RILs population of F8 generation was created via single seed transmission method, which were derived from the cross of barley varieties GP and H602. The developed 134 RILs and parents were planted in the experimental field of Hangzhou Normal University, Xiasha Campus (Hangzhou, Zhejiang province, China) in the order of their numbers, with conventional field cultivation. Each line was sown one row with proximately 30 seeds, 130 cm length and 20 cm row spacing. From 2017 to 2019, the agronomic traits of 10 individuals of each line were measured in March or April.

2.2. Trait Measurement

Using a tape measure or ruler, the PL, AL, NL, LL and LW were measured. Among them, the flag leaf of the main stem was selected to examine the LL and LW, the longest awn was used to quantify AL, and the distance between the flag leaf's petiole and the base of the rachis was used to determine NL. Each trait was measured in 10 plants.

2.3. High-Density Genetic Linkage Map Construction

The specific-locus-amplified fragment sequencing (SLAF-seq) was performed following the description by Fang et al. [17]. All the reads were mapped to the barley reference genome (https://plants.ensembl.org/Hordeum_vulgare/Info/Index, accessed on 20 March 2019) and SLAFs were developed from the RILs and parents. The polymorphic SLAFs were classified into seven linkage groups (LGs). Due to the massive SNP data, a high

density genetic map was constructed by HighMap mapping software (https://mybiosoftware.com/highmap-construction-and-analysis-of-high-density-linkage-map.html#google_vignette, accessed on 20 March 2019), and the genetic distances between two adjacent markers were computed in each LG.

2.4. Data and QTL Analysis

The descriptive statistics and correlative analysis of five traits were performed using SPSS 20.0 software (International Business Machines Corp, Armonk, NY, USA), and the frequency distributions of traits were analyzed using GraphPad Prism 9.5 (http://www.graphpad-prism.cn, accessed on 4 June 2024). Based on the high-density genetic map, QTL analysis was performed using the QGene 4.4.0 software [18]. During the process, the data of each trait were uploaded, and scan interval was set to one milli Morgan. Then, the composite interval mapping model was adopted for scanning seven chromosomes. The logarithm of odds (LOD) threshold greater than 3.5 was considered for significant differences (p = 0.05), and each QTL interval was tested by 1000 permutations. Finally, the QTLs were named obeying the rules presented by Mccouch et al. [19], and then QTLs, QTL parameters of chromosomes, marker names and intervals were obtained for drawing localization mapping using MapChart [20].

3. Results

3.1. Statistical Analysis of Measurement Data

Five traits of 134 RILs and two parents were measured in 3 consecutive years, and the data were statistically analyzed. The results demonstrated that the parent GP showed decreased AL, NL, LL and LW than H602. However, compared to H602, the PL of GP was decreased in 2017 and 2018, and increased in 2019. Except for PL, the average values of the AL, NL, LL and LW of 134 RILs were all between the parents. In addition, continuous segregation and transgressive segregation were observed for all assayed traits in 134 RILs (Table 1 and Figure 1), indicating that QTLs affecting individual's phenotype might come from two parents. Furthermore, the absolute values of skewness and kurtosis of each trait were less than 1, and the plots of the normal distribution revealed a single peak, indicating that the data fit the normal distribution model and were suitable for QTL mapping analysis.

Table 1. Descriptive statistics of parents' and RIL population's agronomic traits.

Year	Traits	GP	H602	RILs		Skewness	Kurtosis
		Mean ± SD (cm)	Mean±SD (cm)	Range (cm)	Mean ± SD (cm)		
2017	PL	7.17 ± 0.15	10.10 ± 0.75 *	5.23–12.97	8.61 ± 1.58	0.36	−0.17
	AL	4.97 ± 0.12	8.93 ± 0.52 **	1.97–111.5	6.91 ± 2.00	0.13	−0.79
	NL	3.03 ± 0.06	20.60 ± 0.51 **	1.80–28.8	12.00 ± 5.79	0.56	0.20
	LL	9.92 ± 0.79	13.60 ± 0.68 **	5.68–20.12	11.32 ± 3.10	0.51	0.21
	LW	0.88 ± 0.05	1.14 ± 0.05 **	0.58–1.58	0.99 ± 0.18	0.42	0.58
2018	PL	8.10 ± 0.21	8.47 ± 0.27	5.43–12.33	8.51 ± 1.35	0.21	−0.37
	AL	6.23 ± 0.34	9.97 ± 0.03 **	5.03–12.87	8.64 ± 1.70	0.01	−0.61
	NL	0.00	15.57 ± 0.25 **	0.00–21.83	9.41 ± 5.20	0.19	−0.82
	LL	6.17 ± 0.47	7.87 ± 0.41 *	3.1–12.43	7.27 ± 2.07	0.56	−0.09
	LW	0.62 ± 0.04	0.78 ± 0.04	0.30–1.43	0.72 ± 0.20	0.82	0.79
2019	PL	8.58 ± 0.23	8.14 ± 0.21	5.32–11.86	8.94 ± 1.23	0.01	−0.11
	AL	6.14 ± 0.15	10.16 ± 0.32 **	5.14–13.58	9.58 ± 1.97	−0.38	−0.59
	NL	0.00	20.5 ± 0.73 **	0.00–23.44	11.70 ± 5.75	0.10	−0.68
	LL	7.16 ± 0.51	9.96 ± 0.63 **	4.72–13.56	8.84 ± 2.12	0.10	−0.91
	LW	0.58 ± 0.09	0.9 ± 0.17 **	0.42–1.32	0.79 ± 0.20	0.52	0.08

GP, Golden Promise; RILs, recombinant inbred lines; PL, panicle length; AL, awn length; NL, neck length; LL, flag leaf length; LW, flag leaf width. Mean ± SD indicates the means and the standard deviation; "*" indicates significant difference between parents; "**" indicates a highly significant difference between parents.

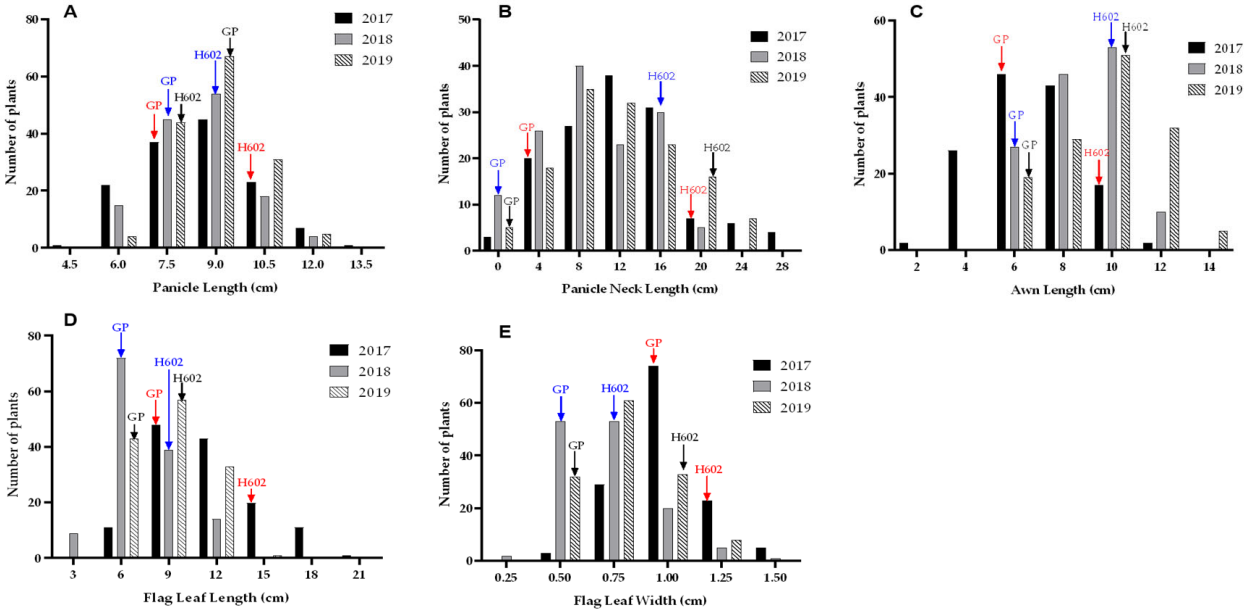

Figure 1. Frequency distribution of agronomic traits in the RIL population. (**A**–**E**) are the frequency distribution of panicle lengthe, panicle neck length, awn length, flag leaf length and flag leaf width, respectively. The *X*-axis represents the length in centimeters, and the *Y*-axis represents the number of plants. Red, blue and black arrows represent the location of the parental GP and H602 in 2017, 2018 and 2019, respectively. RIL, recombinant inbred line; GP, golden promise.

3.2. Correlation Analysis

The Spearman correlation coefficients between the PL, AL, NL, LL and LW of RILs in 3 consecutive years were analyzed by SPSS 20.0. It was found that LL versus LW exhibited extremely significant positive correlations in 3 consecutive years, and the most correlation coefficients of LL versus PL, LL versus AL, LL versus NL and LW versus NL showed significant or extremely significant positive correlations, indicating that correlation traits may be affected by the same genes or may have a strong linkage effect. The correlation data are presented in Table 2.

Table 2. Spearman correlation analysis among different traits in the RIL population.

	PL17	PL18	PL19	AL17	AL18	AL19	NL17	NL18	NL19	LL17	LL18	LL19	LW17	LW18	LW19
PL17	1														
PL18	0.72 **	1													
PL19	0.66 **	0.74 **	1												
AL17	0.35 **	0.09	0.05	1											
AL18	0.27 **	0.17	0.08	0.65 **	1										
AL19	0.20 *	0.10	0.09	0.57 **	0.70 **	1									
NL17	0.24 **	−0.11	−0.05	0.29 **	0.17 *	0.18 *	1								
NL18	0.06	0.04	0.02	−0.09	0.04	−0.02	0.59 **	1							
NL19	0.09	0.03	0.16	−0.03	0.06	0.20 *	0.52 **	0.62 **	1						
LL17	0.32 **	0.08	0.02	0.32**	0.19 *	0.09	0.31 **	0.14	0.14	1					
LL18	0.22 *	0.30 **	0.19 *	0.28 **	0.19 *	0.18*	0.21 *	0.39 **	0.33 **	0.54 **	1				
LL19	0.27 **	0.22 *	0.22 *	0.19 *	0.06	0.22**	0.26 **	0.28 **	0.46 **	0.45 **	0.56 **	1			
LW17	0.20 *	0.133	0.04	0.01	−0.14	−0.18*	0.15	0.21 *	0.14	0.76 **	0.48 **	0.42 **	1		
LW18	0.09	0.21 *	0.08	0.07	−0.10	−0.06	0.18 *	0.41 **	0.30 **	0.41 **	0.80 **	0.45 **	0.64 **	1	
LW19	0.10	0.16	0.06	−0.02	−0.22 *	−0.07	0.18 *	0.34 **	0.37 **	0.27 **	0.42 **	0.77 **	0.53 **	0.62 **	1

17, 18 and 19 were the abbreviations of 2017, 2018 and 2019, respectively; PL, panicle length; AL, awn length; NL, neck length; LL, flag leaf length; LW, flag leaf width. "*" indicates significant difference between traits; "**" indicates a highly significant difference between traits.

3.3. QTL Analysis

Through analysis, a total of 32 QTLs were detected in the 3 years (Table 3 and Figure 2), which were localized on chromosomes 2H, 3H, 4H, 5H, 6H and 7H. Their LOD values ranged from 3.85 to 20.16, and contribution to phenotypic variation ranged from 12.4 to 50%. Among them, the QTL *qAL5* detected in 2017 exhibited the highest LOD value of 20.16 and a contribution rate of 50%. The additive effects of QTLs were positive, indicating that the favorable alleles were derived from the parental H602. Among the 32 QTLs, *qAL5*, *qNL2*, *qNL3*, *qNL6*, *qNL7*, *qPL2*, *qLL3*, *qLW2* and *qLW3* were detected in three consecutive years, and showed high contribution rates, revealing that these QTLs are stably inherited and are less affected by the external environment and other factors.

Table 3. QTL analysis of agronomic traits in the RIL population.

Year	QTLs	Chr	Interval (cM)	Peak LOD	R^2 (%)	Effect	Flank Markers	Physical Pos. (Mb)
2017	*qAL5*	5	58.9–65.6	20.16	50	1.406	Marker5580080–Marker6842284	467.44–488.28
	qNL2	2	0–8.4	9.01	26.6	2.978	Marker24990312–Marker23545719	63.53–64.08
	qNL3	3	41.8–48.2	7.4	22.4	2.736	Marker19746494–Marker21929437	374.11–477.74
	qNL4	4	21.3–23.5	4.4	14	2.162	Marker14966870–Marker11037594	338.34–401.70
	qNL5	5	35.1–41.5	7.95	23.9	2.849	Marker5710486–Marker6018261	354.49–416.31
	qNL6	6	38.8–47.4	13.79	37.7	3.655	Marker1551125–Marker2570397	303.29–480.82
	qNL7	7	69.1–75.9	8.57	25.5	2.94	Marker8576789–Marker8377405	153.50–211.47
	qPL2	2	103.9–107.3	7.05	21.5	0.736	Marker24884231–Marker24834355	730.26–743.57
	qPL5	5	61.9–65.5	6.41	19.8	0.701	Marker6998274–Marker6842284	481.62–488.28
	qLL5	5	53.6–58.1	4.48	14.3	1.159	Marker3862529–Marker4756954	446.24–462.05
	qLW2	2	0–3.8	4.94	15.6	0.072	Marker24990312–Marker26781447	46.72–236.45
	qLW3	3	38–44.8	7.21	21.9	0.086	Marker21428815–Marker18993774	173.69–408.378
2018	*qAL5*	5	61.5–67.2	11.39	33.30	0.978	Marker4410393–Marker5466564	481.70–488.67
	qNL2	2	0–4.9	10.87	31.2	2.906	Marker24990312–Marker25394564	46.72–236.45
	qNL3	3	41.4–47	11.87	33.5	3.019	Marker19120814–Marker21365319	287.83–458.84
	qNL6	6	38.1–43.9	4.67	14.9	2.009	Marker1438212–Marker2973766	303.29–430.94
	qNL7	7	59.4–63.3	7.79	23.5	2.527	Marker8501384–Marker10200503	54.37–127.93
	qPL2	2	103.9–108.1	5.48	17.2	0.559	Marker24884231–Marker23702882	730.26–747.92
	qLL3	3	38.7–42.5	3.86	12.4	0.726	Marker20887162–Marker18512482	253.66–416.32
	qLW2	2	0–2.2	3.85	12.4	0.071	Marker24990312–Marker24565213	46.72–236.45
	qLW3	3	38.7–43.3	6.32	19.5	0.089	Marker20887162–Marker21996421	173.69–416.32
2019	*qAL5*	5	61.5–67.2	6.45	19.9	0.877	Marker4410393–Marker5466564	481.70–488.67
	qNL2	2	0–3.8	11.1	31.7	3.237	Marker24990312–Marker26781447	46.72–236.45
	qNL3	3	41.4–47.8	11.05	31.6	3.235	Marker19120814–Marker18774403	374.11–479.05
	qNL6	6	37.3–40.7	4.32	13.8	2.129	Marker1066730–Marker1300046	295.15–390.93
	qNL7	7	59.4–63.3	7.01	21.4	2.662	Marker8501384–Marker10200503	54.37–127.93
	qPL2	2	103.9–107.3	6.38	19.7	0.545	Marker24884231–Marker24834355	730.26–743.57
	qLL2	2	0–3.8	5.16	16.2	0.856	Marker24990312–Marker26781447	46.72–236.45
	qLL3	3	40.6–46.3	5.26	16.5	0.857	Marker18862742–Marker18763097	287.83–444.09
	qLL7	7	61.4–65	4.22	12.3	0.746	Marker10083213–Marker9339689	59.29–127.93
	qLW2	2	0–2.2	6.11	18.9	0.085	Marker24990312–Marker24565213	46.72–236.45
	qLW3	3	39.1–43.6	5.19	16.3	0.079	Marker21868285–Marker19438077	253.66–416.32

Chr represents the chromosome number; peak LOD represents the maximum likelihood LOD value; R2 (%) represents the contribution rate of phenotypic variation; effect indicates additive effect, and the additive effect is positive, indicating that the favorable allele comes from H602, on the contrary, it comes from GP.

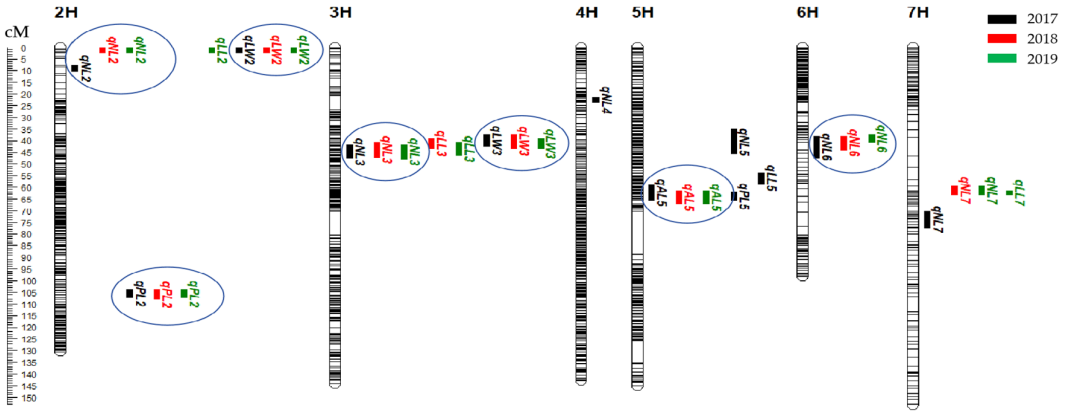

Figure 2. Localization of 32 QTLs for barley yield on the genetic linkage map. Black, red and green boxes represent QTLs in 2017, 2018 and 2019, respectively. Circles represent QTLs detected in 3 consecutive years. Black lines indicate SLAF markers.

3.3.1. QTL Analysis for Awn Length

From 2017 to 2019, the awn length-related QTL *qAL5* on chromosome 5H was detected in 3 consecutive years, which showed LOD values from 6.45 to 20.16 and phenotypic variance from 11% to 50%. Among them, the maximum LOD value of *qAL5* detected in 2017 was 20.16, and the phenotypic variance was as high as 50%. This result indicated that *qAL5* from parental H602 was a major QTL with a high contribution rate and stable inheritance, rendering it suitable for genetic improvement.

3.3.2. QTL Analysis for Panicle Neck Length

A total of 14 QTLs of panicle neck length were identified on chromosomes 2H, 3H, 4H, 5H, 6H and 7H. The LOD values of these QTLs ranged from 4.32 to 13.79, and the contribution to phenotypic variance ranged from 13.8% to 37.7%. Four QTLs named *qNL2*, *qNL3*, *qNL6* and *qNL7* were detected in 3 consecutive years. In 2017, the maximum LOD value of *qNL6* was detected to be 13.79, and its contribution to phenotypic variation reached 37.7%. In 2018 and 2019, the contribution rates of *qNL2* and *qNL3* were detected to be greater than 30%. However, the *qNL4* and *qNL5* were only detected in 2017, and the LOD values and phenotypic variation rate were relatively small, indicating that these two QTLs should be minor genes and are susceptible to environmental factors.

3.3.3. QTL Analysis for Panicle Length

A total of four QTLs for panicle length trait were detected on chromosomes 2H and 5H. The LOD values ranged from 5.48 to 7.05 and the contribution to phenotypic variation ranged from 17.2% to 21.5%. The *qPL2* was detected in all 3 years and the localization intervals were highly overlapped, suggesting that *qPL2* is a major and stable genetic QTL locus for panicle length. Moreover, the *qPL5* was only detected in 2017 and explained the phenotypic variance of 17.2%, indicating that it was highly susceptible to environmental impacts.

3.3.4. QTL Analysis for Flag Leaf Length and Width

According to the result of QTL analysis for flag leaf length, five QTLs were identified, which were localized on chromosomes 2H, 3H, 5H and 7H with LOD values ranging from 3.86 to 5.26 and contribution to phenotypic variation ranging from 12.4 to 16.5%. Among them, the *qLL3* was detected in 2018 and 2019, *qLL2* and *qLL7* were detected in 2019 and *qLL5* was only detected in 2017. None of the flag leaf length QTLs could be mapped in 3 consecutive years, indicating that they were greatly affected by environmental factors. In flag leaf width, a total of six QTLs were detected, which were localized on chromosomes 2H and 3H. Among them, the *qLW2* and *qLW3* were all detected in 3 consecutive years with

the LOD values ranged from 3.85 to 6.32 and a phenotypic variation of 12.4% to 19.5%, revealing that leaf width can be stably inherited.

4. Discussion

The yield of gramineous crops is determined by both genetic and environmental factors. Therefore, the key to increasing yield is by selecting high heritability gene loci. The panicle length can directly affect the yield, whereas the panicle neck length and awn length indirectly affect the yield by resisting diseases and pests [21]. The flag leaf is the topmost leaf, and its size is determined by length and width, which influences photosynthesis rate to a certain extent, thereby affecting crop yield [22]. Although yield-related QTLs have been extensively studied in various crops, such as rice (*Oryza sativa* L.) [23–25], wheat (*Triticum aestivum* L.) [26,27] and maize (*Zea mays* L.) [28,29], the related research progress is relatively limited in barley.

There is a strong correlation between panicle length and yield. Longer panicles usually produce more seeds, which will be beneficial for improving the harvest index. Previous QTL studies of panicle length in barley have mainly focused on chromosome 2H. Chutimanitsakun et al. [30] identified a QTL on chromosome 2H that significantly affects various traits, including panicle length, plant height and panicle kernels. Similarly, Xue et al. [31] identified a QTL with close location for panicle length between markers bPb-6088 and bPb-5440. Moreover, Rozanova et al. [32] also identified three related loci on chromosome 2H. Islamovic et al. [33] positioned a QTL related to panicle length at 102.8 cm on chromosome 2H, which is very close to the localization interval of *qPL2* in the present study, suggesting that they may be controlled by the same QTL.

Barley awn is essentially degraded leaves, which play an important role in protecting plant from exogenous aggression, efficiently dispersing seeds and promoting photosynthetic efficiency [34]. Using a multiparent mapping population, Liller et al. [15] identified 12 QTLs related to awn length, which were distributed on all seven chromosomes of barley, respectively. In our study, a QTL related to awn length, *qAL5*, was mapped to an interval of 58.9–67.2 cM on chromosome 5H, which was close to the position of *qAL5.1* detected by Liller et al. [15], suggesting that the two may be the same QTL. However, only 1 QTL for awn length was detected in 3 consecutive years in this study, indicating that it may be related to the parental material used to construct the population. Considering the high contribution and stable genetic ability of *qAL5*, it can be used for breeding applications.

In this study, no QTL related to panicle leaf length was repeatedly detected in 3 consecutive years, and the contribution to phenotypic variance were also lower that of other trait QTLs, which may be related to the mapped population and environmental factors. However, two QTLs related to panicle leaf width, *qLW2* and *qLW3*, were detected on chromosomes 2 and 3H in 3 consecutive years. In addition, *qLL2* and *qLW2* were mapped to the same interval, and *qLL3* and *qLW3* were also highly overlapped, suggesting that there may be genetic pleiotropy that can control both LL and LW. It has been reported that a QTL on the 2H chromosome can simultaneously influence the length, width, area and chlorophyll content of flag leaves in barley [35]. Similarly, another QTL on chromosome 2H has also been revealed as affecting both the length and width of flag leaves [36]. These studies validate our conclusion that LL and LW are governed by the same gene.

In gramineous plants, there is also a relationship between the panicle neck length and yield. A longer panicle neck length helps to efficiently transport nutrients produced by photosynthesis to the grain, thereby increasing grain filling and yield [37]. However, excessively long panicle neck length directly leads to the increase in plant height, which in turn induces lodging and has a negative effect on yields [38]. To date, there are still only a few reports on QTLs related to panicle neck length in barley. In this study, we identified 14 QTLs associated with panicle neck length. Among them, the *qNL2*, *qNL3* and *qNL6*, located on chromosomes 2H, 3H and 6H, respectively, were detected in 3 consecutive years, while the others were only identified for 1 or 2 years. A previous report revealed that the panicle neck length was easily affected by environmental factors in wheat [39]. By

analyzing the phenotypic data of panicle neck length from 2017 to 2019, we found that the panicle neck length of parent GP and some RIL lines were zero or close to zero in 2018 and 2019, but were significantly elongated in 2017, suggesting that barley panicle neck length may be also easily influenced by the environment. Combined with the high overlap between *qLL*, *qLW* and *qNL* on 2H and 3H chromosomes, we speculate that there may be a locus that regulates leaf morphology and panicle neck length at the same time.

5. Conclusions

In this study, the QTL analysis related to panicle and flag leaf traits was carried out by a high-density genetic map based on 134 RILs. A total of 32 QTLs related to five yield-related traits, panicle length, awn length, panicle neck length and flag leaf length and width were detected, which were distributed on chromosomes 2H to 7H. Among them, *qPL2*, *qLW2*, *qLW3*, *qNL2*, *qNL3*, *qNL6* and *qAL5* were detected in 3 consecutive years and their mapping positions were overlapped, indicating that these QTLs were genetically stable and less affect by environmental factors. These results lay a foundation for molecular breeding of these traits.

Author Contributions: Conceptualization: Y.F., X.Z. and D.X.; methodology: Y.F., X.Z. and D.X.; investigation: Y.Y., S.W., X.Y. and G.Z.; writing—original draft preparation: Y.Y. and S.W.; writing—review and editing: Y.F., X.Z. and D.X. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Specific data from this study can be obtained by contacting the authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Abou-Elwafa, S.F. Association mapping for drought tolerance in barley at the reproductive stage. *Comptes Rendus Biol.* **2016**, *339*, 51–59. [CrossRef] [PubMed]
2. Cossani, C.M.; Palta, J.; Sadras, V.O. Genetic yield gain between 1942 and 2013 and associated changes in phenology, yield components and root traits of Australian barley. *Plant Soil* **2022**, *480*, 151–163. [CrossRef]
3. Du, B.; Wu, J.; Wang, Q.; Sun, C.; Sun, G.; Zhou, J.; Zhang, L.; Xiong, Q.; Ren, X.; Lu, B. Genome-wide screening of meta-QTL and candidate genes controlling yield and yield-related traits in barley (*Hordeum vulgare* L.). *PLoS ONE* **2024**, *19*, e0303751. [CrossRef] [PubMed]
4. Alqudah, A.M.; Schnurbusch, T. Barley leaf area and leaf growth rates are maximized during the pre-anthesis phase. *Agronomy* **2015**, *5*, 107–129. [CrossRef]
5. Ma, J.; Tu, Y.; Zhu, J.; Luo, W.; Liu, H.; Li, C.; Li, S.; Liu, J.; Ding, P.; Habib, A.; et al. Flag leaf size and posture of bread wheat: Genetic dissection, QTL validation and their relationships with yield-related traits. *Theor. Appl. Genet.* **2020**, *133*, 297–315. [CrossRef]
6. Abebe, T.; Wise, R.P.; Skadsen, R.W. Comparative transcriptional profiling established the awn as the major photosynthetic organ of the barley spike while the lemma and the palea primarily protect the seed. *Plant Genome* **2009**, *2*, 247–259. [CrossRef]
7. Abebe, T.; Skadsen, R.W.; Kaeppler, H.F. Cloning and Identification of Highly Expressed Genes in Barley Lemma and Palea. *Crop Sci.* **2004**, *44*, 942–950. [CrossRef]
8. Jian, M.A.; Min, S.; Puyang, D.; Wei, L.; Xiaohong, Z.; Congcong, Y.; Zhang, H.; Qin, N.; Yang, Y.; Lan, X. Genetic Identification of QTL for Neck Length of Spike in Wheat. *J. Triticeae Crops* **2017**, *37*, 319–324.
9. Bridgemohan, P.; Bridgemohan, R.S. Evaluation of anti-lodging plant growth regulators on the growth and development of rice (*Oryza sativa* L.). *J. Clin. Oncol.* **2014**, *5*, 12–16.
10. Elbaum, R.; Zaltzman, L.; Burgert, I.; Fratzl, P. The Role of Wheat Awns in the Seed Dispersal Unit. *Science* **2007**, *316*, 884–886. [CrossRef]
11. Wang, J.; Sun, G.; Ren, X.; Li, C.; Liu, L.; Wang, Q.; Du, B.; Sun, D. QTL underlying some agronomic traits in barley detected by SNP markers. *BMC Genet.* **2016**, *17*, 103. [CrossRef] [PubMed]
12. Varshney, R.K.; Paulo, M.J.; Grando, S.; Eeuwijk, F.V.; Keizer, L.C.; Guo, P.; Ceccarelli, S.; Kilian, A.; Baum, M.; Graner, A. Genome wide association analyses for drought tolerance related traits in barley (*Hordeum vulgare* L.). *Field Crops Res.* **2012**, *126*, 171–180. [CrossRef]
13. Niu, Y.; Chen, T.; Zheng, Z.; Zhao, C.; Liu, C.; Jia, J.; Zhou, M. A new major QTL for flag leaf thickness in barley (*Hordeum vulgare* L.). *BMC Plant Biol.* **2022**, *22*, 305. [CrossRef]

14. Du, B.; Liu, L.; Wang, Q.; Sun, G.; Ren, X.; Li, C.; Sun, D. Identification of QTL underlying the leaf length and area of different leaves in barley. *Sci. Rep.* **2019**, *9*, 4431. [CrossRef] [PubMed]
15. Liller, C.B.; Walla, A.; Boer, M.P.; Hedley, P.; Macaulay, M.; Effgen, S.; von Korff, M.; van Esse, G.W.; Koornneef, M. Fine mapping of a major QTL for awn length in barley using a multiparent mapping population. *Theor. Appl. Genet.* **2017**, *130*, 269–281. [CrossRef] [PubMed]
16. Sato, K. History and future perspectives of barley genomics. *DNA Res.* **2020**, *27*, dsaa023. [CrossRef]
17. Fang, Y.; Zhang, X.; Zhang, X.; Tong, T.; Zhang, Z.; Wu, G.; Hou, L.; Zheng, J.; Niu, C.; Li, J.; et al. A High-Density Genetic Linkage Map of SLAFs and QTL Analysis of Grain Size and Weight in Barley (*Hordeum vulgare* L.). *Front. Plant Sci.* **2020**, *11*, 620922. [CrossRef]
18. Joehanes, R.; Nelson, J.C. Qgene 4.0, an extensible java QTL-analysis platform. *Bioinformatics* **2008**, *24*, 2788–2789. [CrossRef]
19. McCouch, S.R.; Cho, Y.G.; Yano, M.; Paul, E.; Blinstrub, M.; Morishima, H.; Kinoshita, T. Report on QTL nomenclature. *Rice Genet. Newsl.* **1997**, *14*, 11–13.
20. Voorrips, R.E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* **2002**, *93*, 77–78. [CrossRef]
21. Hua, L.; Wang, D.R.; Tan, L.; Fu, Y.; Liu, F.; Xiao, L.; Zhu, Z.; Fu, Q.; Sun, X.; Gu, P.; et al. *LABA1*, a Domestication Gene Associated with Long, Barbed Awns in Wild Rice. *Plant Cell* **2015**, *27*, 1875–1888. [CrossRef] [PubMed]
22. Xue, D.W.; Chen, M.C.; Zhou, M.X.; Chen, S.; Mao, Y.; Zhang, G.P. QTL analysis of flag leaf in barley (*Hordeum vulgare* L.) for morphological traits and chlorophyll content. *J. Zhejiang Univ. Sci. B* **2008**, *9*, 938–943. [CrossRef] [PubMed]
23. Xu, Z.; Li, M.; Du, Y.; Li, X.; Wang, R.; Chen, Z.; Tang, S.; Liu, Q.; Zhang, H. Characterization of *qPL5*: A novel quantitative trait locus (QTL) that controls panicle length in rice (*Oryza sativa* L.). *Mol. Breed.* **2022**, *42*, 70. [CrossRef]
24. Zhao, D.D.; Son, J.H.; Farooq, M.; Kim, K.M. Identification of Candidate Gene for Internode Length in Rice to Enhance Resistance to Lodging Using QTL Analysis. *Plants* **2021**, *10*, 1369. [CrossRef] [PubMed]
25. Wang, P.; Zhou, G.; Yu, H.; Yu, S. Fine mapping a major QTL for flag leaf size and yield-related traits in rice. *Theor. Appl. Genet.* **2011**, *123*, 1319–1330. [CrossRef]
26. Kuang, C.H.; Zhao, X.F.; Yang, K.; Zhang, Z.P.; Ding, L.; Pu, Z.E.; Ma, J.; Jiang, Q.T.; Chen, G.Y.; Wang, J.R.; et al. Mapping and characterization of major QTL for spike traits in common wheat. *Physiol. Mol. Biol. Plants* **2020**, *26*, 1295–1307. [CrossRef]
27. Liu, K.; Xu, H.; Liu, G.; Guan, P.; Zhou, X.; Peng, H.; Yao, Y.; Ni, Z.; Sun, Q.; Du, J. QTL mapping of flag leaf-related traits in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* **2018**, *131*, 839–849. [CrossRef]
28. Yang, J.; Liu, Z.; Chen, Q.; Qu, Y.; Tang, J.; Lübberstedt, T.; Li, H. Mapping of QTL for Grain Yield Components Based on a DH Population in Maize. *Sci. Rep.* **2020**, *10*, 7086. [CrossRef]
29. Zhang, J.; Fengler, K.A.; Van Hemert, J.L.; Gupta, R.; Mongar, N.; Sun, J.; Allen, W.B.; Wang, Y.; Weers, B.; Mo, H.; et al. Identification and characterization of a novel stay-green QTL that increases yield in maize. *Plant Biotechnol.* **2019**, *17*, 2272–2285. [CrossRef]
30. Chutimanitsakun, Y.; Nipper, R.W.; Cuesta-Marcos, A.; Cistué, L.; Corey, A.; Filichkina, T.; Johnson, E.A.; Hayes, P.M. Construction and application for QTL analysis of a Restriction Site Associated DNA (RAD) linkage map in barley. *BMC Genom.* **2011**, *12*, 4. [CrossRef]
31. Xue, D.W.; Zhou, M.X.; Zhang, X.Q.; Chen, S.; Wei, K.; Zeng, F.R.; Mao, Y.; Wu, F.B.; Zhang, G.P. Identification of QTLs for yield and yield components of barley under different growth conditions. *J. Zhejiang Univ. Sci. B* **2010**, *11*, 169–176. [CrossRef] [PubMed]
32. Rozanova, I.V.; Grigoriev, Y.N.; Efimov, V.M.; Igoshin, A.V.; Khlestkina, E.K. Genetic Dissection of Spike Productivity Traits in the Siberian Collection of Spring Barley. *Biomolecules* **2023**, *13*, 909. [CrossRef] [PubMed]
33. Islamovica, E.; Obertc, D.E.; Oliverk, R.E.; Marshall, J.; Miclausd, K.J.; Hanga, A.; Chaof, S.; Lazog, G.R.; Harrisonh, S.A.; Ibrahimi, A.; et al. A new genetic linkage map of barley (*Hordeum vulgare* L.) facilitates genetic dissection of height and spike length and angle. *Field Crops Res.* **2013**, *154*, 91–99. [CrossRef]
34. Huang, B.; Huang, D.; Hong, Z.; Owie, S.O.; Wu, W. Genetic analysis reveals four interacting loci underlying awn trait diversity in barley (*Hordeum vulgare*). *Sci. Rep.* **2020**, *10*, 12535. [CrossRef] [PubMed]
35. Liu, L.; Sun, G.; Ren, X.; Li, C.; Sun, D. Identification of QTL underlying physiological and morphological traits of flag leaf in barley. *BMC Genet.* **2015**, *16*, 29. [CrossRef] [PubMed]
36. Gyenis, L.; Yun, S.J.; Smith, K.P.; Steffenson, B.J.; Bossolini, E.; Sanguineti, M.C.; Muehlbauer, G.J. Genetic architecture of quantitative trait loci associated with morphological and agronomic trait differences in a wild by cultivated barley cross. *Genome* **2007**, *50*, 714–723. [CrossRef]
37. Li, C.; Tang, H.; Luo, W.; Zhang, X.; Mu, Y.; Deng, M.; Liu, Y.; Jiang, Q.; Chen, G.; Wang, J.; et al. A novel, validated, and plant height-independent QTL for spike extension length is associated with yield-related traits in wheat. *Theor. Appl. Genet.* **2020**, *133*, 3381–3393. [CrossRef]
38. Berry, P.M.; Sylvester-Bradley, R.; Berry, S. Ideotype design for lodging-resistant wheat. *Euphytica* **2007**, *154*, 165–179. [CrossRef]
39. Jia, Y.; Zhang, Y.; Sun, Y.; Ma, C.; Bai, Y.; Zhang, H.; Hou, J.; Wang, Y.; Ji, W.; Bai, H.; et al. QTL Mapping of Yield-Related Traits in Tetraploid Wheat Based on Wheat55K SNP Array. *Plants* **2024**, *13*, 1285. [CrossRef]