

## Article

# Quantitative Trait Loci Mapping of First Pod Height in Faba Bean Based on Genotyping-by-Sequencing (GBS)

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**Abstract:** Candidate genes related to first pod height (FPH) traits in faba bean plants are crucial for mechanised breeding. However, reports on quantitative trait locus (QTL) mapping related to the FPH of faba bean are few, thus limiting the high-quality development of the faba bean industry to a certain extent. The identification and screening of candidate genes related to FPH is extremely urgent for the advancement of mechanised breeding for faba bean. In this study, a high-density genetic linkage map was constructed using genotyping-by-sequencing (GBS) of an F<sub>3</sub> population and QTLs (genes) related to FPH were identified. The genetic linkage map contained seven linkage groups with 3012 SNP markers with an overall length of 4089.13 centimorgan (cM) and an average marker density of 1.36 cM. Thirty-eight QTLs for the first pod node (FPN) and FPH were identified (19 each for FPN and FPH). The 19 QTLs associated with FPN were located on chromosomes 1L, 1S, 2, 3, 4, 5, and 6; the 19 QTLs associated with FPH were located on chromosomes 1L, 1S, 2, 3, 5, and 6. There was a co-localisation interval of *qFPN6-1* and *qFPH6-1* on chromosome 6. By annotating the QTL *qFPH6-1* interval, 36 genes that may be related to FPH were identified, these genes are related to plant growth and development. The results provide a basis for the precise location of QTLs related to FPH and could accelerate the breeding of faba bean varieties adapted to mechanised harvesting.

**Keywords:** faba bean; FPH; GBS; genetic linkage map; QTL mapping; candidate genes



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## 1. Introduction

Faba bean (*Vicia faba* L.) is a widely planted cold-season legume crop and an important legume crop with abundant protein, vitamins, and mineral nutrients. Therefore, faba bean consumption can help maintain a balanced diet [1,2]. Faba bean can be applied as green manure because of its effective nitrogen fixation effect, providing approximately 90% of the plant nitrogen demand and increasing soil fertility and crop yield during crop rotation with other crops [3]. It also plays an important role in the sustainable development of the agricultural economy [4]. Faba bean is a diploid plant (2n = 12) with a “giant genome” (approximately 13 Gb) [5], which restricts the progress of genetic linkage mapping, quantitative trait loci (QTL) mapping, and gene mining [6]. Therefore, basic research on faba bean is not as advanced as that on other crops with well-defined genomes, such as *Oryza sativa* L., *Glycine max* L., and *Triticum aestivum* L. [7].

Molecular markers have been widely applied in botanical research to construct genetic maps, QTL location, marker-assisted breeding, species phylogenetic relationships, and genetic diversity analysis [8,9]. The first faba bean molecular linkage map was constructed by Van De Ven et al. [10]. Recently, significant progress has been made in identifying molecular markers of faba bean. It is beneficial to construct high-density genetic maps and contribute to the gene mining of faba bean using single-nucleotide polymorphism (SNP) markers [11,12]. For example, Sudheesh et al. [13] combined a published genetic map with

a newly constructed genetic map based on SNP markers and constructed an integrated map containing six linkage groups and 1850 markers. The total coverage length was 1439 cM, and the average marker density was 0.8 cM. This study identified two QTLs for resistance to ascochyta blight of faba bean and explained a total of up to 49% of phenotypic variance. Carrillo-Perdomo et al. [14] constructed a map incorporating 1728 markers using 1819 SNP markers that exhibited polymorphism in three  $F_3$  populations; the length of the faba bean genetic map is 1547.71 cM, with an average genetic distance of 0.89 cM. This was a SNP marker developed based on transcriptome sequencing. Li et al. [15] constructed an integrated SNP molecular map of 6895 SNPs with an overall length of 3324.48 cM from two  $F_2$  populations. A total of 98 QTLs for 14 agronomic traits related to the flowers, pods, plant types, and grains were identified. Zhao et al. [16] constructed the first genetic map of ultra-high-density faba bean, containing 12,023 SNP markers with a length of 1182.65 cM and an average distance of 0.098 cM. A total of 65 QTLs for seed-related traits were identified. High-quality genetic map construction for the faba bean can considerably accelerate related gene studies and breeding processes. It can establish molecular marker development and QTL locations related to economically important traits [17,18]. The faba bean genome contains 13 billion bases, more than four times that of the human genome. An article published in *Nature* on 8 March 2023, reported the first high-quality, chromosome-sized genome of faba bean, which is crucial for functional research and cloning of faba bean genes [19].

Currently, the cultivation of leguminous crops generally relies on mechanised harvesting, which limits the production of leguminous crops. Therefore, breeding optimised varieties suitable for mechanised harvesting has become a critical challenge for breeders [20]. FPH is an important trait that is relevant for mechanically harvested varieties. Varieties with lower FPH may be overcut, destroyed, or lost during mechanised harvesting [21]. Zdravkovic et al. [22] studied a genetic model and found that the genes related to FPH were recessive. FPH is an important factor affecting yield in soybean breeding and is negatively correlated with plant density and nitrogen application [23]. Recently, few studies have been conducted on QTL mapping of leguminous crops for FPH. Jiang et al. [21] conducted a QTL mapping analysis on 147 recombinant inbred lines (RILs) and identified 11 major QTLs based on the molecular maps of 5308 SLAF markers. Among these, eight candidate genes controlled soybean FPH, and seven candidate genes were highly expressed in soybean stems. Research on QTL location in faba bean has mainly focused on biological and abiotic stresses, including brown spot resistance and cold tolerance [11,24]. Other studies have mainly focused on QTL mapping of agronomic characteristics in faba bean associated with flowers, pods, plant types, and seeds [15,16]. However, reports on QTL mapping related to the FPH of faba bean are limited.

The aim of this study was to construct a high-density SNP genetic linkage map with the  $F_3$  population (Qinghai13  $\times$  Qingcan19) using GBS. This technology is beneficial for exploring related markers and their applications in faba bean breeding.

## 2. Materials and Methods

### 2.1. Population and Phenotypic Data Measurement

The female parent, Qinghai13, had a low FPN and FPH, whereas the male parent had a high FPN and FPH. The  $F_1$  population was generated by crossing 'Qinghai13' and 'Qingcan19'. The parents and  $F_1$  progeny were planted in 2021, and  $F_2$  seeds were produced. All the  $F_2$  seeds were planted for observation of phenotypic traits in 2022, and  $F_3$  family seeds were thus produced. In 2023, the parents and  $F_3$  populations were planted in three environments, including Xining (XN, 36.72° N, 101.75° E, 2309 m), Gonghe (GH, 36.23° N, 99.57° E, 2880 m), and Huzhu (HZ, 36.84° N, 101.96° E, 2550 m) in Qinghai Province, China. Due to the low reproductive rate of faba bean, the number of seeds per plant is relatively small. Each variety was planted in a row with 10–12 plants, with no duplicates. Field management was performed according to the planting standards of the native region throughout the growing season. At the mature stage, the FPN and FPH of the  $F_3$  population

and parents were measured. FPN is the node where the lowest pod on the main stem is located [25]. FPH is the distance from the ground to the bottom of the first pod over the cotyledon node [21].

## 2.2. DNA Extraction and SNP Genotyping

At the seedling stage, leaves were collected from the parental and F<sub>3</sub> populations for DNA extraction. The leaves were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ .

DNA was extracted using a CretMag Power Plant Pro DNA Kit (CretBiotech Co. Ltd., Suzhou, China). GBS technology (Oebiotech Co. Ltd., Shanghai, China) was used to develop SNP markers. The restriction endonuclease selection of GBS is carried out by predicting the fragment sizes produced by various enzyme combinations, and a combination of *MseI* and *NlaIII* was selected to construct the GBS library. Each sample was subjected to multiple amplifications, and the required fragments were selected for library construction.

The raw data were analysed using the performance standard. Dual-ended, i.e., paired-end (PE), 150 sequencing was performed using the Illumina HiSeq TM sequencing platform (Illumina, San Diego, CA, USA). Based on the analysis of raw data, advanced analysis and assembly of the DNA library were performed, and HiSeq sequencing was performed to eliminate low-quality readings due to the presence or absence of base calls [26].

To ensure the reliability of the reads, three strict filtering criteria were used to filter the original reads, removing the following: (1) reads with unrecognised nucleotides  $\geq 10\%$ , (2) reads with a Phred quality score  $< 5$   $> 50\%$  bases, and (3) reads with more than 10 nucleotides aligned with the adapter, allowing for  $\leq 10\%$  mismatch.

Finally, in the offspring GBS analysis, we analysed the number of reads cleaved by *MseI* at both ends of each screened read; reads that did not include these restrictive inlier sites were discarded [26]. Finally, GBS analysis of the later generation was performed to analyse the number of *MseI* reads at each end of the filter reading and discard the reading without these limiting cut parts. Simultaneously, we recorded the ratio of specific and total reads to enzyme-captured reads. The selected tags were subjected to end-to-end sequencing using the Illumina high-throughput sequencing platform (Oebiotech Co. Ltd., Shanghai, China), followed by SNP genotyping and evaluation.

## 2.3. Statistical Analysis

The phenotypic data were analysed using SPSS 27.0 software (SPSS, Chicago, IL, USA) to generate descriptive statistics, including the mean, minimum, maximum, standard deviation (SD), coefficient of variation (CV), skewness, and kurtosis. The frequency distributions of phenotypic data were checked using SPSS 27.0 software as well. The kurtosis and skewness were used to estimate the frequency distribution normality [27], and the correlation between FPN and FPH was obtained with Pearson's correlation analyses using SPSS 27.0 software.

## 2.4. Comparison with Reference Genome

The parents and 242 offsprings were sequenced and compared with a reference genome to perform GBS. The Burrows–Wheeler aligner (BWA) [26,28] was used to compare the readings of the parents and F<sub>3</sub> generations with the faba bean genome. Gene sequences were compared with reference genomics (Hedin2 genome v1) in the gene spectra (<https://projects.au.dk/fabagenome/genomics-data> accessed on 2 March 2024).

## 2.5. Genetic Linkage Map Construction

The comparison of multiple types of fuel tanks for Qinghai13  $\times$  Qingcan19 F<sub>3</sub> yielded a consistent  $\chi^2$  score greater than 10, and these were excluded from the analysis ( $p < 0.05$ ). Before constructing a genetic map, genotype screening was performed on the harvested samples. According to the screened genotypes, the individual codes for each locus were “A” (same as parent Qinghai13), “B” (same as parent Qingcan19), “H” (heterozygotes that contain two alleles from each parent), or “missing” (all other cases). The discarded loci

included (1) loci that were heterozygous in either parent or (2) loci with a missing rate above 20% in the population [29]. Abandoned gene loci include (1) genetic loci in which one of the parents is mixed and (2) loci of gene loss in the population [16]. QTL IciMapping (version 4.2) was used to construct a genetic linkage map [30].

The additive effect of QTLs on a single allele was measured using the inclusive composite interval mapping (ICIM) add plot (ICIM-ADD) function. Using 1000 sequences, type I error determined the logarithm of the remarkable QTL odds (LOD) value to be less than 0.05. The step size of the QTL scan was one cM, and the step regression (PIN) probability was 0.001. Subsequently, a visual link graph was drawn using MapChart 2.2 [31].

## 2.6. QTL Mapping

Based on the phenotypic data and genetic linkage maps, the BIP function of the QTL positioning of the separated population was determined using QTL IciMapping Software (version 4.2) [15,30]. The positioning method includes the ICIM and sets the LOD detection threshold to 2.5 [32,33]. The QTL name was constructed as follows: q + trait name + LG or LG number + “-” + QTL number.

## 2.7. Candidate Gene Prediction for the QTLs of FPH

The faba bean genome was released in 2023, facilitating the annotation of 44,410 genes [19]. The candidate genes were homologously compared with *Arabidopsis thaliana*, *Glycine max* L., and *Medicago truncatula*. Genes were annotated within the QTL interval to screen candidate genes related to the growth and development of faba bean.

## 3. Results

### 3.1. Phenotypic Analysis and Correlation Analysis

Statistical analysis was performed using SPSS 27.0. The FPN and FPH phenotypic data of Qinghai13, Qingcan19, and F<sub>3</sub> populations in the three environments (Table 1) showed that the two traits were significantly different between parents, and the FPN and FPH of Qingcan19 were significantly higher than those of Qinghai13. The range of FPN in the F<sub>3</sub> population in the three environments is 3.00–11.50, and the range of FPH is 8.33–63.25. The average value of the F<sub>3</sub> population tended to be low, and the coefficient of variation of the three environments was 22.99–38.70%. This indicated that there was a large variation in FPN and FPH in the F<sub>3</sub> population. The normality test results showed a  $p < 0.05$ , and the F<sub>3</sub> population followed a skewed normal distribution in all three environments, which was consistent with the genetic characteristics of quantitative traits and was suitable for QTL mapping analysis (Table 2 and Figure 1). The correlation between FPN and FPH in the three environments was studied, with values of 0.857 \*\*, 0.871 \*\*, and 0.826 \*\*, respectively. Two traits showed highly significant correlations with each other.

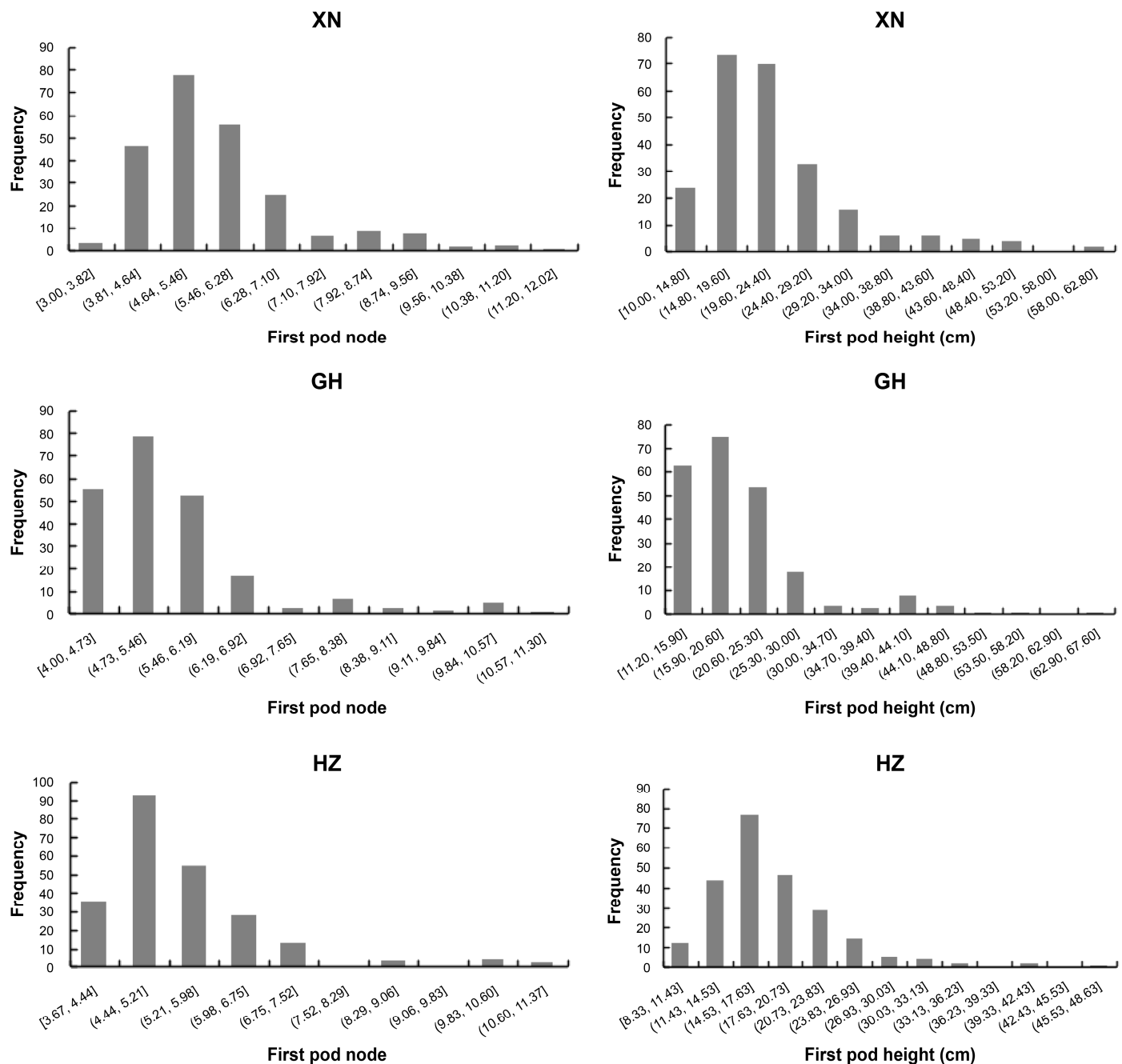
**Table 1.** Phenotypic data of FPN and FPH based on the F<sub>3</sub> populations in the three environments.

Trait	Envn.	Parents		F <sub>3</sub> Population							h <sup>2</sup> /%
		Qinghai13	Qingcan19	Min	Max	Mean	SD	CV/%	Skewness	Kurtosis	
FPN	XN	5.11 **	9.70 **	3.00	11.50	5.67	1.45	25.57	1.44	2.30	87.67
	GH	5.00 **	9.40 **	4.00	11.00	5.48	1.26	22.99	2.14	5.24	
	HZ	5.20 **	10.40 **	3.67	11.25	5.46	1.37	25.09	2.07	5.08	
FPH/cm	XN	27.89 **	64.65 **	10.00	61.00	22.95	8.59	37.43	1.72	3.59	81.53
	GH	17.70 **	46.20 **	11.20	63.25	21.37	8.27	38.70	2.12	5.37	
	HZ	14.90 **	39.10 **	8.33	47.50	18.11	5.55	30.65	1.56	4.59	

Env, environment; Min, minimum; Max, maximum; SD, Standard deviation; h<sup>2</sup>, broad-sense heritability; CV, coefficient of variation (%); \*\* denotes the significance of the parents at the 0.01 level.

**Table 2.** Tests of normality in F<sub>3</sub> populations in three environments.

Envn.	Kolmogorov–Smirnov			Shapiro–Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
XN	0.168	240	<0.001	0.881	240	<0.001
GH	0.195	232	<0.001	0.783	232	<0.001
HZ	0.177	241	<0.001	0.798	241	<0.001



**Figure 1.** Frequency distribution of FPN and FPH based on F<sub>3</sub> population in three environments.

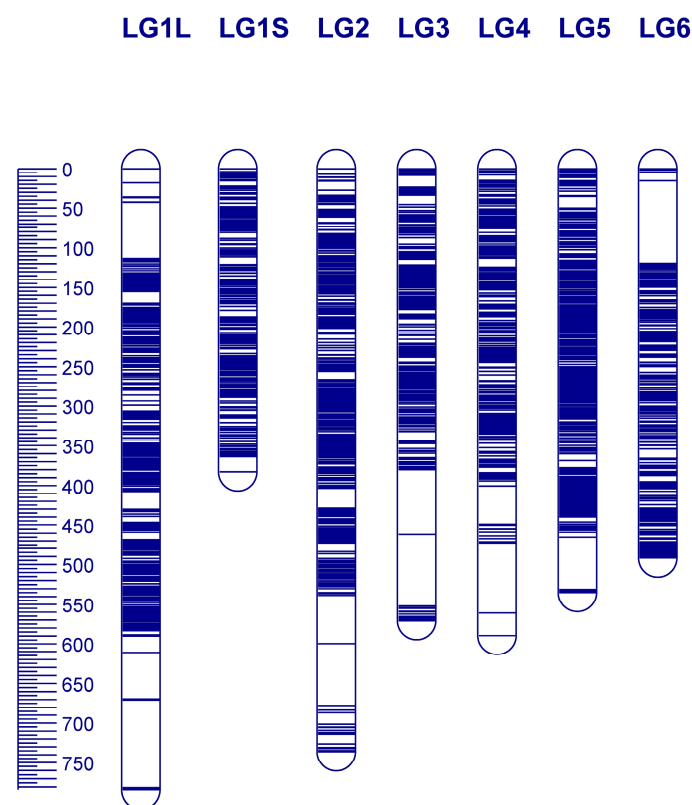
### 3.2. Genotyping-by-Sequencing

In total, 1,716,981,024 clean reads were obtained from 242 individuals, 12,180,704 clean reads were obtained from the maternal parent, ‘Qinghai13’, and 12,398,160 clean reads were obtained from the paternal parent, ‘Qingcan19’. The number of clean reads obtained

from the 244 samples ranged from 5.31 to 12.40 M, with an average of 7.14 M reads (Supplementary Materials), and 99.23% of the clean reads were matched with the faba bean genome.

### 3.3. Construction of High-Density Genetic Map

The F<sub>3</sub> population contained 11,340 SNP markers after screening, of which 3012 SNP markers were obtained via progeny and heterozygous screening and were selected to construct a genetic linkage map. SNP filtering was performed using the ‘SNP’ and ‘BIN’ functions of QTL IciMapping (version 4.2). After partial separation, filtration, and chain distribution, 3012 markers covered seven LGs, corresponding to seven chromosomes of faba bean (Figure 2).



**Figure 2.** High-density genetic linkage map of faba bean.

The overall length of the genetic map was 4089.13 cM, and the average marker density was 1.36 cM. LG2 had the maximum number of markers at 565 SNPs. The genetic distance of LG1L was the longest (783.26 cM), whereas the shortest length of LG1S was 382.64 cM and 335 SNPs (Table 3). During the assembly of the faba bean genome, because of the length of chromosome 1, it was divided into long and short arms, resulting in two LGs.

**Table 3.** Distribution of high-density SNP markers in the linkage groups.

Chr	SNP	Genetic Distance (cM)	Mean Gap (cM)	Max. Gap (cM)
1L	509	783.26	1.54	110.40
1S	335	382.64	1.14	18.85
2	565	736.17	1.30	77.81
3	363	570.82	1.57	90.60
4	355	589.40	1.66	88.06
5	546	534.95	0.98	66.19
6	339	491.89	1.45	104.08
Total	3012	4089.13	1.36	



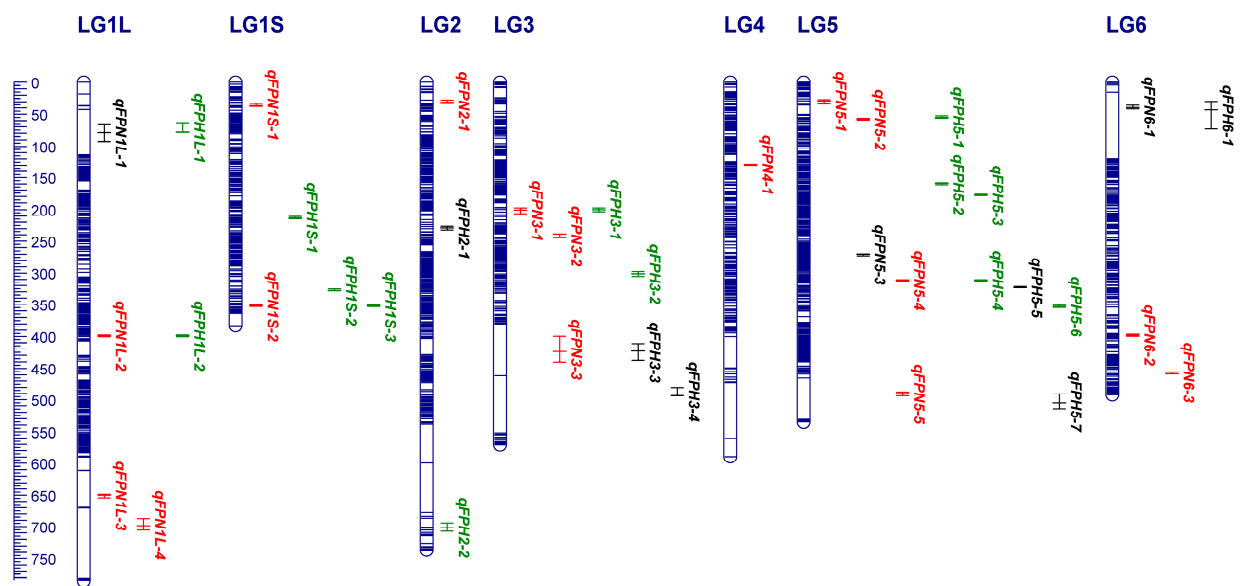
All SNP markers were anchored to seven LGs, with 3005 gaps between adjacent markers. Among them, 2038 gaps were less than 1 cM, and 886 gaps were within the range of  $1 \text{ cM} < D < 5 \text{ cM}$ . Additionally, 2924 gaps were less than 5 cM, with a ratio of  $>97\%$ , and 2038 gaps were less than 1 cM, with a ratio of  $>67\%$  (Table 4). The total number of gaps greater than 20 cM was 15, and the maximum gap was 110.40 cM in LG1L. Additionally, each genetic linkage map had gaps greater than 15 cM, thereby increasing the genetic distance between markers and the overall length of the map.

**Table 4.** Distribution of SNP marker intervals on the genetic linkage groups.

Chr	<1 (cM)	1–5 (cM)	<5 (cM)	5–10 (cM)	10–20 (cM)	>20 (cM)	Ratio <5 (cM)	Ratio <1 (cM)
1L	359	133	492	8	3	5	96.85	70.67
1S	220	106	326	7	1	0	97.60	65.87
2	389	158	547	9	5	3	96.99	68.97
3	250	99	349	8	3	2	96.41	69.06
4	211	131	342	8	1	3	96.61	59.60
5	391	146	537	6	1	1	98.53	71.74
6	218	113	331	4	2	1	97.93	64.50
Total	2038	886	2924	50	16	15	97.30	67.82

### 3.4. QTL Mapping of FPN and FPH

Using the ICIM method, a total of 38 QTLs related to FPN and FPH of faba bean were mapped on seven chromosomes in three environments. There were 19 QTLs associated with FPN and FPH, and nine QTLs were detected repeatedly in two or more environments, involving eight chromosome segments (Table 5 and Figure 3).



**Figure 3.** Locations of quantitative trait loci in LGs. FPN QTLs are in red, and FPH QTLs are in green. QTLs identified in two or more environments are shown in black.

The 19 QTLs associated with FPN were located on chromosomes 1L, 1S, 2, 3, 4, 5, and 6. Two important QTLs (*qFPN1L-1* and *qFPN6-1*) on chromosomes 1L and 6 were detected in three environments, with maximum LOD values of 6.54 and 7.39, respectively, and the maximum phenotypic variation rates that could be explained were 12.37% and 12.47%, respectively. The *qFPN5-3* on chromosome 5 was detected repeatedly in two environments, with the maximum LOD value and phenotypic contribution rate of 7.21% and 1.7%, respectively. The 19 QTLs associated with FPH were located on chromosomes 1L, 1S, 2, 3, 5, and 6. Six QTLs were detected in two environments, and the maximum LOD

value and phenotypic contribution rate were 11.21% and 13.44%, respectively (Table 5). Nine QTLs were detected in two or more environments, five of which were in larger marker intervals. There was a co-localisation interval of *qFPN6-1* and *qFPH6-1* on chromosome 6, which was located between markers chr6\_SNP630 and chr6\_SNP8, and the genetic distance was 15.44–119.52 cM. Ultimately, we selected *qFPH6-1* for subsequent analysis.

**Table 5.** Quantitative trait loci analysis of FPN and FPH in the F<sub>3</sub> population.

Trait	Chr.	QTL	Env.	Pos./cM	Marker Interval	Genetic Distance/cM	LOD	PVE/%	Add
FPN	1L	<i>qFPN1L-1</i>	GH	79.0	chr1L_SNP937–chr1L_SNP1943	65.5–92.5	3.36	0.89	0.02
			HZ	83.0	chr1L_SNP937–chr1L_SNP1943	67.5–94.5	7.39	5.83	−0.01
			XN	87.0	chr1L_SNP937–chr1L_SNP1943	68.5–98.5	3.25	12.37	−0.04
		<i>qFPN1L-2</i>	XN	399.0	chr1L_SNP1308–chr1L_SNP1302	397.5–400.5	4.22	2.41	−0.40
			HZ	650.0	chr1L_SNP81–chr1L_SNP243	648.5–654.5	6.37	5.81	−0.01
			HZ	699.0	chr1L_SNP249–chr1L_SNP1011	687.5–705.5	7.91	5.84	0.01
	1S	<i>qFPN1S-1</i>	XN	36.0	chr1S_SNP138–chr1S_SNP154	32.5–37.5	2.50	1.34	0.23
			XN	352.0	chr1S_SNP1127–chr1S_SNP1133	351.5–353.5	5.31	3.16	0.49
			HZ	31.0	chr2_SNP1828–chr2_SNP1816	27.5–32.5	2.57	0.57	−0.30
	3	<i>qFPN3-1</i>	GH	201.0	chr3_SNP664–chr3_SNP645	197.5–206.5	2.58	0.67	0.19
			XN	239.0	chr3_SNP540–chr3_SNP526	238.5–244.5	2.73	1.59	0.35
			GH	424.0	chr3_SNP4–chr3_SNP113	399.5–440.5	5.38	6.59	−0.07
	4	<i>qFPN4-1</i>	XN	129.0	chr4_SNP1080–chr4_SNP1062	128.5–130.5	4.15	2.30	0.34
	5	<i>qFPN5-1</i>	HZ	29.0	chr5_SNP1020–chr5_SNP996	26.5–33.5	2.63	0.55	0.07
			GH	58.0	chr5_SNP956–chr5_SNP966	56.5–59.5	3.07	0.80	0.04
			GH	270.0	chr5_SNP436–chr5_SNP435	269.5–272.5	6.49	1.70	0.43
		<i>qFPN5-2</i>	HZ	270.0	chr5_SNP436–chr5_SNP435	269.5–272.5	7.21	1.55	0.54
			XN	311.0	chr5_SNP305–chr5_SNP304	310.5–312.5	12.39	7.55	0.77
			GH	490.0	chr5_SNP148–chr5_SNP1096	487.5–493.5	2.93	5.18	−0.05
	6	<i>qFPN6-1</i>	HZ	39.0	chr6_SNP630–chr6_SNP8	35.5–41.5	2.92	4.07	−0.01
			XN	41.0	chr6_SNP630–chr6_SNP8	30.5–73.5	3.57	12.47	0.04
			GH	41.0	chr6_SNP630–chr6_SNP8	31.5–70.5	6.54	6.63	0.08
		<i>qFPN6-2</i>	XN	398.0	chr6_SNP1256–chr6_SNP1266	396.5–399.5	4.43	2.55	−0.01
			XN	458.0	chr6_SNP1460–chr6_SNP1466	455.5–458.5	3.54	1.98	−0.11
			HZ	79.0	chr1L_SNP937–chr1L_SNP1943	63.5–78.5	2.97	5.02	0.12
FPH	1L	<i>qFPH1L-1</i>	XN	399.0	chr1L_SNP1308–chr1L_SNP1302	397.5–400.5	7.11	5.35	−3.36
			HZ	213.0	chr1S_SNP636–chr1S_SNP635	210.5–214.5	3.28	0.69	1.06
			GH	328.0	chr1S_SNP1026–chr1S_SNP1038	325.5–328.5	2.95	0.34	2.08
	1S	<i>qFPH1S-1</i>	XN	352.0	chr1S_SNP1127–chr1S_SNP1133	351.5–353.5	6.46	4.88	3.20
			HZ	229.0	chr2_SNP940–chr2_SNP916	226.5–232.5	2.85	0.67	0.31
			XN	230.0	chr2_SNP940–chr2_SNP916	225.5–233.5	2.54	1.73	0.94
	2	<i>qFPH2-1</i>	XN	702.0	chr2_SNP1857–chr2_SNP1869	694.5–707.5	2.54	1.77	1.46
			GH	200.0	chr3_SNP664–chr3_SNP645	197.5–203.5	2.70	0.30	1.10
			XN	301.0	chr3_SNP407–chr3_SNP404	297.5–305.5	2.71	1.85	1.37
	3	<i>qFPH3-1</i>	GH	423.0	chr3_SNP4–chr3_SNP113	411.5–437.5	9.89	3.32	−0.09
			HZ	431.0	chr3_SNP4–chr3_SNP113	417.5–443.5	2.90	5.11	−0.22
			HZ	491.0	chr3_SNP113–chr3_SNP1599	480.5–493.5	2.93	5.04	−0.49
		<i>qFPH3-2</i>	GH	528.0	chr3_SNP113–chr3_SNP1599	526.5–533.5	10.04	3.35	−0.80
			XN	55.0	chr5_SNP971–chr5_SNP958	52.5–56.5	5.21	3.87	−0.45
			XN	159.0	chr5_SNP704–chr5_SNP701	158.5–161.5	5.46	3.94	−2.78
	5	<i>qFPH5-1</i>	HZ	178.0	chr5_SNP655–chr5_SNP651	176.5–178.5	2.69	0.60	0.34
			XN	311.0	chr5_SNP305–chr5_SNP304	310.5–312.5	12.46	9.76	4.57
			GH	323.0	chr5_SNP277–chr5_SNP278	321.5–323.5	3.08	0.35	2.08
		<i>qFPH5-2</i>	HZ	323.0	chr5_SNP277–chr5_SNP278	321.5–323.5	6.03	1.37	1.92
			XN	353.0	chr5_SNP234–chr5_SNP222	350.5–354.5	2.51	1.77	−0.76
			GH	505.0	chr5_SNP148–chr5_SNP1096	490.5–514.5	10.58	3.41	−0.99
	6	<i>qFPH5-3</i>	HZ	507.0	chr5_SNP148–chr5_SNP1096	494.5–517.5	2.97	4.95	−0.09
			XN	43.0	chr6_SNP630–chr6_SNP8	29.5–73.5	2.62	13.44	0.34
			GH	46.0	chr6_SNP630–chr6_SNP8	33.5–52.5	11.21	3.35	0.61
		<i>qFPH5-4</i>	GH	63.0	chr6_SNP630–chr6_SNP8	61.5–71.5	10.88	3.34	0.52

Chr, chromosome number; Env, environment; Pos, position; PVE, phenotypic variation rate; Add, additive effect.



### 3.5. Mining of Candidate Genes

A total of 1657 genes were screened in the *qFPH6-1* interval. Through gene annotation, 36 genes that may be related to FPH were screened (Table 6). These genes involve plant hormones and signal transduction pathways, including auxin response factors and proteins, serine, and threonine-protein kinases, which are related to plant growth and development. The results provide a basis for the study of FPH candidate genes and the breeding of faba bean varieties adapted to mechanised harvesting.

**Table 6.** Annotation of candidate genes.

Gene ID	Annotation
<i>Vfaba.Hedin2.R1.6g005240.1</i>	Phosphatase 2c
<i>Vfaba.Hedin2.R1.6g010640.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g010760.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g010960.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g017960.1</i>	Belongs to the protein kinase superfamily. Ser Thr protein kinase family
<i>Vfaba.Hedin2.R1.6g018200.1</i>	Serine threonine-protein kinase Nek5-like
<i>Vfaba.Hedin2.R1.6g018240.1</i>	Serine threonine-protein kinase Nek5-like
<i>Vfaba.Hedin2.R1.6g026360.1</i>	Auxin-responsive protein
<i>Vfaba.Hedin2.R1.6g027040.1</i>	Receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g027480.1</i>	Auxin response factors (ARFs) are transcriptional factors that bind specifically to the DNA sequence 5'-TGTCTC-3' found in the auxin-responsive promoter elements (AuxREs)
<i>Vfaba.Hedin2.R1.6g028040.1</i>	Auxin response factors (ARFs) are transcriptional factors that bind specifically to the DNA sequence 5'-TGTCTC-3' found in the auxin-responsive promoter elements (AuxREs)
<i>Vfaba.Hedin2.R1.6g032960.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g037560.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g040280.1</i>	May act as a component of the auxin efflux carrier
<i>Vfaba.Hedin2.R1.6g040680.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g042000.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g042600.1</i>	Serine threonine-protein kinase-like protein
<i>Vfaba.Hedin2.R1.6g045520.1</i>	Inactive leucine-rich repeat receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g046360.1</i>	Auxin transporter-like protein
<i>Vfaba.Hedin2.R1.6g047520.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g048160.1</i>	Auxin-induced protein
<i>Vfaba.Hedin2.R1.6g048200.1</i>	Auxin-induced protein
<i>Vfaba.Hedin2.R1.6g048640.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g048920.1</i>	Auxin-responsive protein
<i>Vfaba.Hedin2.R1.6g053960.1</i>	Belongs to the protein kinase superfamily. Ser Thr protein kinase family
<i>Vfaba.Hedin2.R1.6g056240.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g058160.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g062480.1</i>	Auxin-responsive protein
<i>Vfaba.Hedin2.R1.6g064920.1</i>	Serine threonine-protein kinase sepA-like
<i>Vfaba.Hedin2.R1.6g065000.1</i>	Serine threonine-protein kinase sepA-like
<i>Vfaba.Hedin2.R1.6g067520.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g071920.1</i>	Auxin response factors (ARFs) are transcriptional factors that bind specifically to the DNA sequence 5'-TGTCTC-3' found in the auxin-responsive promoter elements (AuxREs)
<i>Vfaba.Hedin2.R1.6g075760.1</i>	Checkpoint serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g079880.1</i>	Serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g086080.1</i>	G-type lectin S-receptor-like serine threonine-protein kinase
<i>Vfaba.Hedin2.R1.6g087120.1</i>	Belongs to the protein kinase superfamily. Ser Thr protein kinase family

## 4. Discussion

### 4.1. High-Density Genetic Linkage Map of Faba Bean

There are many methods for constructing genetic maps based on SNPs, such as restrictive site-associated DNA sequencing (RAD-seq), invented by Baird et al. [34], genotyping-by-sequencing [35], specific length amplification fragment sequencing (SLAF-seq) [36], and targeted next-generation sequencing [37]. Four high-density genetic linkage maps were constructed for faba bean (Table 7). High-density genetic linkage maps are based

on transcriptome data or liquid-phase gene chips developed using transcriptome data for genotyping. The faba bean genome is currently the largest leguminous plant to be sequenced and contains many non-coding DNA sequences. Although the faba bean genome is complex, the number of genes is relatively small (approximately 44,410), indicating that the functional regions of the faba bean genome are relatively concentrated, making it easier for researchers to conduct relevant research [19]. Recently, a high-density map of faba bean was constructed using 12,023 SNP markers based on the Faba\_bean\_130 K-targeted next-generation sequencing genotyping platform [16]. This map was the first ultra-dense genetic map for the faba bean with an average gap of <0.1 cM (0.098 cM) between adjacent markers. This map's quantity, density, and distribution quality were significantly improved compared with the genetic maps used by previous researchers. When comparing the positions of chromosomes and genes in the faba bean reference genome, only approximately 60% of the genes in each LG were located on the corresponding chromosomes. Due to the large genome size of faba bean, the cost of re-sequencing is very high, and applying GBS can help reduce the price of high-density genetic maps [38]. In the present study, we used GBS technology to screen for SNP markers and constructed a map by comparing it with the faba bean reference genome. A genetic linkage map with a length of 4089.13 cM was constructed using 3012 SNP markers, which was longer than that used in previous studies; however, the marker distribution was relatively uniform. The linkage map had 15 gaps greater than 20 cM, possibly due to random enzyme digestion in GBS-simplified sequencing. The gap ratio of less than 5 cM exceeded 97%, and the gap ratio of less than 1cM exceeded 67%. The quality of the constructed map was high and suitable for QTL mapping. This resulted in significant missing data for some markers due to low sequencing depth and insufficient genome coverage. Because the resequencing of faba bean is expensive, it is a good choice to use GBS for QTL mapping. We hope our results will provide some reference significance for fine mapping, gene mining, and cloning.

**Table 7.** High-density genetic linkage map based on SNP markers in faba bean.

No.	Name of Parent	Number of Progenies	Number of Markers	Map Length (cM)	Average Distance (cM)	LG	References
1	Nura 9 × Farah	F <sub>4</sub> (145)	1850	1439	0.8	6	[13]
2	HIVERNA, NOVA GRADISKA, SILIAN, and QUASAR	F <sub>3</sub> (345)	1728	1547.71	0.89	6	[14]
3	Yundou 8137, H0003712, H000572	F <sub>2</sub> (371)	6895	3324.48	0.48	6	[15]
4	WY7 × TCX7	F <sub>2</sub> (121)	12,023	1182.65	0.098	6	[16]

#### 4.2. Analysis of QTL for FPN and FPH

The distance from the bottom soil of the plant to the first pod or the FPH is a crucial characteristic when using a mechanical combination harvester [39,40]. When the FPH is too low, the joint harvesting opportunity is cut off, causing damage, or even missing the bottom pod and decreasing grain yield and resulting in unnecessary losses. Furthermore, pod height affects the quality of bottom pods. Pods that are too low and come in contact with soil in high-humidity environments can decay and are affected by diseases and pests [22]. Compared to other agronomic traits of faba bean, most breeders did not initially consider FPH important, and research on FPH is limited. However, with the development of agricultural mechanisation, the importance of FPH has become increasingly apparent.

Consequently, FPH has gradually become an important trait for breeders. Pod height has also become a criterion for measuring the suitability of faba bean varieties for mechanised harvesting and examining whether they can be widely promoted. Cultivating excellent varieties of faba bean suitable for mechanical harvesting is an urgent requirement for breeders. Identifying QTLs related to FPH is important for guiding faba bean molecular-assisted breeding, improving mechanical harvesting efficiency, and ultimately increasing faba bean yield.

Reports on the QTLs of FPH have mainly focused on soybean and common bean, with very few studies focusing on faba bean. Liang et al. [41] tested QTLs on the main agronomic traits of 447 RIL populations. They detected five QTLs highly correlated with FPH in four linkage groups in A2, C2, J\_1, and J\_2. Yuan [42] constructed a genetic map based on 138 RIL strains and conducted QTL testing on soybean FPH traits. Three QTLs related to FPH were detected in the D1b linkage group. Cheng [43] used composite interval mapping (CIM) to conduct a QTL analysis of FPH traits in RIL populations and detected 11 major QTLs in six linkage groups. Jiang et al. [21] conducted a QTL analysis on the 8-year base FPH data of 147 RILs and obtained 11 QTLs related to soybean FPH on five chromosomes. Delfini et al. [44] used the GBS method and detected four quantitative trait nucleotides (QTN) related to FHP in 178 Brazilian common beans, which showed a phenotypic variation (PVE) of 3.03–10.05%.

Ávila et al. [45] constructed a genetic map using the RIL population and found four QTLs for FPH in faba bean located on four different chromosomes. Li et al. [15] identified 10 QTLs for FPN using two F<sub>2</sub> populations in six LGs. QTLs associated with FPH were located in the two populations in LG2, LG5, and LG6. In our study, 19 QTLs associated with FPN were located on chromosomes 1L, 1S, 2, 3, 4, 5, and 6, and 19 QTLs associated with FPH were located on chromosomes 1L, 1S, 2, 3, 5, and 6. FPN and FPH are also within a common interval between LG1 and LG6 [15]. We speculate that a single QTL might control traits related to FPN and FPH. This is consistent with the results of Li et al. [15], with a QTL controlling FPH on chromosome 6.

This study provides useful benchmark information concerning high-density genetic mapping and QTL identification related to the FPH of the faba bean. This will accelerate fine mapping and cloning of related genes. The limitations of this study include the limited number of markers obtained via GBS and the inadequate density of the constructed genetic map. However, except for a few large gaps, the marker density was relatively uniform, which holds a substantial reference value for future QTL mapping and gene mining.

#### 4.3. Candidate Genes Associated with FPH of Faba Bean

Currently, there is no report on the FPH gene of faba bean. To screen candidate genes related to FPH in faba bean, we annotated 1657 genes in *qFPH6-1* intervals and observed that 36 genes may be involved in the formation of FPH. Eight genes were identified to be associated with FPH in soybean, and four were related to signal transduction pathways. These includes auxin response factors, small auxin (AUX) up RNA (SAUR) family proteins, serine/threonine protein kinases, and transmembrane amino acid transporter family proteins. The homologous genes of these genes in *Arabidopsis* are involved in plant growth and development pathways, indicating that FPH may be regulated by AUX [21]. The FPH is directly related to the number of nodes and the length of internodes. The length of internodes is related to the process of cell elongation and division, and the length of internodes is determined by elongation [46]. The number of nodes of the first flower (or pod) is not the only factor that determines the height of the first pod. It affects the height of the first pod together with the length of the internode [47]. The number of nodes and internode length were also regulated by AUX [48,49], and AUX had a negative regulatory effect on the formation of soybean pods [50]. Plant height and internode length could also be controlled by other genes related to GA signal transduction [46]. Research has shown that gibberellin plays an important role in internode elongation, and a decrease in gibberellin content or inhibition of related gene synthesis can lead to internode shortening and reduced HFP [51,52]. A study on peas shows that gibberellin and auxin work together to cause internode elongation [53]. A recent report demonstrated that overexpression of *GmGAMYB*, an R2R3-MYB transcription factor, promoted plant height and elongated internodes in soybean plants via positive regulation of the GA-biosynthesis gene *GmGA20ox* [54]. Research has indicated that the regulatory mechanism of mutual antagonism between jasmonic acid and brassinosteroids during maize internode elongation and overexpression

of *ZmbHLH154* resulted in longer internodes [55]. Therefore, our next task is to study the role of gibberellin synthesis-related genes in controlling internode elongation.

## 5. Conclusions

In this study, we constructed a high-density linkage map of faba bean using SNP markers obtained via GBS. The genetic linkage map consisted of 3012 SNP markers covering 4089.13 cM, with an average distance between adjacent markers of 1.36 cM. Based on this genetic map, 38 QTLs were identified for FPN and FPH, including 19 QTLs each for FPN and FPH. Through gene annotation of the co-location *qFPH6-1* interval, 36 genes that may be related to FPH were identified.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14092013/s1>, Table S1: Distribution of read numbers in the parents and F<sub>3</sub> populations; Table S2: Genotyping by sequencing in the parents and F<sub>3</sub> populations.

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