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Identification of Genetic Loci Associated with Bolting Time in Radish (*Raphanus sativus* L.) by QTL Mapping and GWAS

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Abstract: Bolting time is a critical trait that affects crop yield, adaptability, and overall productivity, making its regulation vital for agricultural success. In this study, we explored the genetic mechanisms controlling flowering time in radish (*Raphanus sativus*) via a combination of quantitative trait locus (QTL) analysis and genome-wide association study (GWAS). By developing an F_2 population from a cross between the relatively late-bolting variety 'L432' and the early-bolting variety 'L285', we identified 12 QTLs associated with bolting time. Furthermore, a GWAS performed on 60 East Asian radish accessions revealed 14 candidate genes potentially involved in flowering and bolting regulation. *FLOWERING LOCUS C* (*FLC2*) was the major candidate gene explaining the early and late bolting types. One locus was commonly detected from QTL and GWAS on chromosome 4, where *CONSTANS-like* (*COL4*) is located. To validate these findings, SNP markers were designed and applied to F_2 populations, revealing a correlation between marker presence and bolting phenotypes. These results offer valuable insights into the molecular control of bolting time in radish and identify candidate genes for use in marker-assisted breeding. These findings could enhance breeding efforts for optimizing bolting time in various radish markets.

Keywords: radish; bolting time; QTL; GWAS

Citation: Han, K.; Ahn, H.-I.; Yang, H.-B.; Lee, Y.-R.; Lee, E.-S.; Lee, J.; Jang, C.-S.; Kim, D.-S. Identification of Genetic Loci Associated with Bolting Time in Radish (*Raphanus sativus* L.) by QTL Mapping and GWAS. *Agronomy* **2024**, *14*, 2700. https://doi.org/10.3390/agronomy14112700

Academic Editors: Ainong Shi and Yingpeng Han

Received: 20 September 2024
Revised: 13 November 2024
Accepted: 14 November 2024
Published: 16 November 2024

1. Introduction

Radishes (*Raphanus sativus*) are cultivated with a variety of root colors and shapes worldwide, which are tailored to each country's climate and preferences. Particularly in East Asia, including China, Japan, and Korea, large, long, and white-rooted varieties are highly favored. Depending on the sowing season, radishes can be classified as fall radishes, which are sown in the fall and harvested before winter, and spring radishes, which are sown in the spring and harvested before summer. During the growth period of radishes, the transition from the vegetative stage to the bolting and flowering stage is a crucial phase in the plant's life cycle. The timing of bolting and flowering must be appropriately regulated to ensure reproductive success under favorable conditions [1,2]. Various environmental changes, such as prolonged exposure to low temperatures or an increase in day length, can cause premature bolting. The bolting of plants is induced by various signals, including temperature, photoperiod, autonomous pathways, and hormones [3–5]. Like other Brassicaceae plants, radishes experience accelerated bolting when exposed to prolonged cold, a process known as vernalization [6].

The mechanism that triggers bolting and flowering has been extensively studied, particularly in the model plant *Arabidopsis thaliana*. The *FLC* (*FLOWERING LOCUS C*) gene is a key repressor of flowering, maintaining high expression during the vegetative stage to prevent the transition to flowering. Vernalization and the autonomous pathways reduce

FLC expression, thus enabling flowering [7–9]. *FRI* (*FRIGIDA*) promotes *FLC* expression upstream, and FLC suppresses floral integrator genes such as *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*) and *FT* (*FLOWERING LOCUS T*) [10–12]. These integrators activate downstream genes such as *AP1* and *LFY*, initiating flowering [13]. Vernalization reduces *FLC* expression, releasing the repression of *SOC1* and *FT*, which allows flowering, especially under long-day conditions [14,15]. Furthermore, *VRN2* plays a role in chromatin remodeling to maintain *FLC* repression after the cold period, whereas the *VIN3*/*VRN5* complex recruits the *PRC2* complex to *FLC*, leading to its methylation and epigenetic silencing [8,16,17]. This integrated regulatory network ensures the precise control of flowering timing in response to internal and environmental signals, as demonstrated by various studies in different plant species.

Research on bolting and flowering mechanisms in radish (*Raphanus sativus*) has also been reported, particularly concerning QTL studies. *FLC* is a key gene that suppresses flowering, and its expression decreases after prolonged cold exposure, promoting flowering [18]. Genes such as *FLC*, *SOC1*, *CO*, and *FT* play important roles in integrating environmental signals to regulate the timing of flowering [19,20]. Additionally, genes such as *VIN3* and *VRN2* are involved in chromatin modification, which helps maintain *FLC* repression, and studies have been conducted on how these epigenetic mechanisms regulate flowering in radishes. Recent reports have also shown that gibberellins regulate the expression of the *FT* and *SOC1-1* genes [21]. One study identified QTLs related to bolting time, namely, *qBT2* and *qBT7.2*, which are located on chromosomes 2 and 7. *FLC* and *FRI* are located at these loci and are believed to play critical roles in the response to environmental signals, similar to flowering regulation [21]. Additionally, QTLs related to genes such as *VRN*, *FLC*, and *VIN* on chromosomes 1, 6, and 9 have also been reported [19,22–24].

As many previous studies have indicated, the formation of bolting in radishes is thought to result not from a single dominant gene but from multiple genes, leading to quantitative differences in traits. Therefore, we conducted this experiment to investigate the QTLs related to the genes involved in bolting in late- and early-bolting radish varieties.

2. Materials and Methods

2.1. Plant Materials and Evaluation of Bolting Time

The inbred line ‘L432’ is a relatively late bolting-type radish with white and small roots, whereas ‘L285’ is an early bolting type with round and red-skinned roots (Figure 1). F_2 populations were developed by crossing ‘L432’ and ‘L285’ for QTL analysis of bolting time. The parental lines and F_2 seeds were sown in 50-cell trays and kept in a greenhouse under normal growth conditions (14 October to 14 November). Four-week-old seedlings were transferred to a cold chamber (4–5 °C) for 2 or 3 weeks. The plants were moved to the greenhouse, and the bolting time (days from the end of vernalization until the main stalk reached 4 cm) was measured.

For GWAS analysis, we selected 60 Asian radish varieties (20, 22, and 18 from Korea, China, and Japan; Table S1). Korean varieties were mostly white-skin radishes, except three varieties (R39–R41). Nine of the twenty-two Chinese varieties had special traits including green skin color or red flesh color. Most of the Japanese varieties were white-skin radishes, and nine of the varieties were from the same company (R20–R29). Based on the bolting time information provided by seed companies, we classified the varieties into two types: early bolting and late bolting. The 32 early-bolting varieties included autumn radish, which has high root quality, and Chinese green- and red-skin radishes. Late-bolting varieties are mostly grown in the winter and spring seasons.

Figure 1. Parental lines of F_2 populations used for QTL analysis. (**A**) Inbred lines 'L432' and 'L285'. (**B**) F_2 populations in a cold chamber for vernalization treatment.

2.2. DNA Extraction and Genotyping F_2 and GWAS Populations

DNA was extracted from the cotyledons of the seedlings via the CTAB method [25]. The extracted DNA was stored at −20 °C until use. A genotyping-by-sequencing (GBS) library of parental lines and the F_2 population was constructed using the restriction enzyme ApeKI [26]. A resequencing library of the GWAS population was constructed via a TruSeq Nano DNA Kit. The raw sequences were produced via an Illumina system with a 150 bp paired-end sequencing protocol.

The quality of the raw sequencing data was checked via FastQC [27], and the data were trimmed with a Phred score of 20 and a minimum length of 120 bp via Trimmomatic version 0.3238 [28]. Trimmed sequences were aligned to the 'Okute-Sakurajima' genome v1.0 [29] via BWA version 0.7.8-r455 [30], and variants were called via GATK version 3.1 [31]. Significant SNPs were filtered via an in-house script with a minimum read depth of 5 and a calling rate of 0.6. For the GBS data of the F_2 population, SNPs that were polymorphic between parental lines and had a 1:2:1 segregation ratio (p value of the chi-square test > 0.01) of maternal homozygous, heterozygous, and paternal homozygous F_2 individuals were also selected.

2.3. QTL and GWAS for Bolting Time

A linkage map of the F_2 population was constructed via bin markers investigated by sliding window approach with a window length of 2 Mbp [32]. Using the linkage map constructed by Carthagene [33] and the bolting time phenotype of F_2, QTLs were identified. The composite interval mapping function with the default option was performed via WinQTL Cartographer, and the logarithm of odds (LOD) threshold was determined via a permutation test (500 times with a p value of 0.05) (version 2.5 [34]). The physical positions of the QTLs were detected based on the linked bin markers.

Genotypes of filtered SNPs and binary phenotypes (early and late bolting) were used for GWAS. The population structure was estimated from 3538 randomly selected SNPs (4% of all variants) via the STRUCTURE program (version 2.3.4 [35]). The best K was detected following Kim et al. [36]. GWAS was conducted via GAPIT (version 3, [37]) via the GLM, MLM, and BLINK models with default settings.

2.4. Identification of Candidate Genes and Development of Markers

The physical positions of the QTL and SNPs detected via GWAS were compared based on the reference genome. Annotated genes located on QTL were compared with previously reported genes related to bolting time [19,20,38–40]. The CDSs of the candidate genes detected from the QTL and transcriptome analyses were BLAST searched against the 'Okute-Sakurajima' genome v1.0.

To validate the QTL and GWAS results, high-resolution melting (HRM) markers were developed from SNPs linked to the *RsBT4.3* locus and polymorphic between 'L432' and 'L285'. With the Primer3 program(version 4.1.0), forward and reverse primers were selected to amplify 100–150-bp fragments, including SNPs. HRM was conducted with a total reaction volume of 10 µL containing 100 ng of DNA, 5 pmol of each primer, and 5 µL of qPCRBIO HRM Mix (PB20.31-05, PCR Biosystems Ltd., London, UK). PCR and HRM were performed via a LightCycler 96 (Roche®, Basel, Switzerland) instrument following the manufacturer's protocol. F_2 individuals were analyzed for maternal (A), paternal (B), and heterozygous (H) genotypes.

3. Results

3.1. Variation in Bolting in the F_2 Population

Bolting of the seedlings was induced by vernalization for 1, 2, 3, or 4 weeks. The bolting times of each treatment were 57, 51, 39, and 31 days for 'L432' and 38, 28, 20, and 19 days for 'L285' (Figure S1). L432 showed more late bolting than L285 did in all the treatments, which we called the relatively late bolting type. The difference in bolting time between the parental lines was the greatest at the 2nd and 3rd weeks of vernalization. The differences were 22.8 and 19.6 days from 2 and 3 weeks, respectively. Therefore, the bolting time of the F_2 population was investigated after 2 and 3 weeks of vernalization. The bolting time varied from 21 to 83 days, with an average of 37 days for 2 weeks of treatment, whereas it was between 12 and 53 days for 3 weeks of treatment (Figure 2; Tables S2 and S3). Both distribution graphs showed positive skew and transgressive segregation, indicating that bolting time is a quantitative trait controlled by multiple QTLs.

3.2. Construction of the Bin Map and QTL Analysis

Parental lines ('L432' and 'L285') and 188 F_2 individuals from 2- and 3-week vernalization treatments were genotyped via GBS. A total of 10,275 and 13,041 significant SNPs were detected from each treatment, and a bin map was constructed to reduce the effects of missing data and genotyping errors (Table S4). Every chromosome consisted of 81–207 bin markers with average sizes of 279 kbp (1.5 cM) and 329 kbp (1.1 cM) from the F_2 population cold-treated for 2 and 3 weeks, respectively. The genotypes of the SNPs and bin markers are listed in Tables S5–S8. The bin markers were named chromosomes, and the physical location was Mbp. For example, Bin1_1.5–1.8 is located on 1.5–1.8 Mbp on chromosome 1.

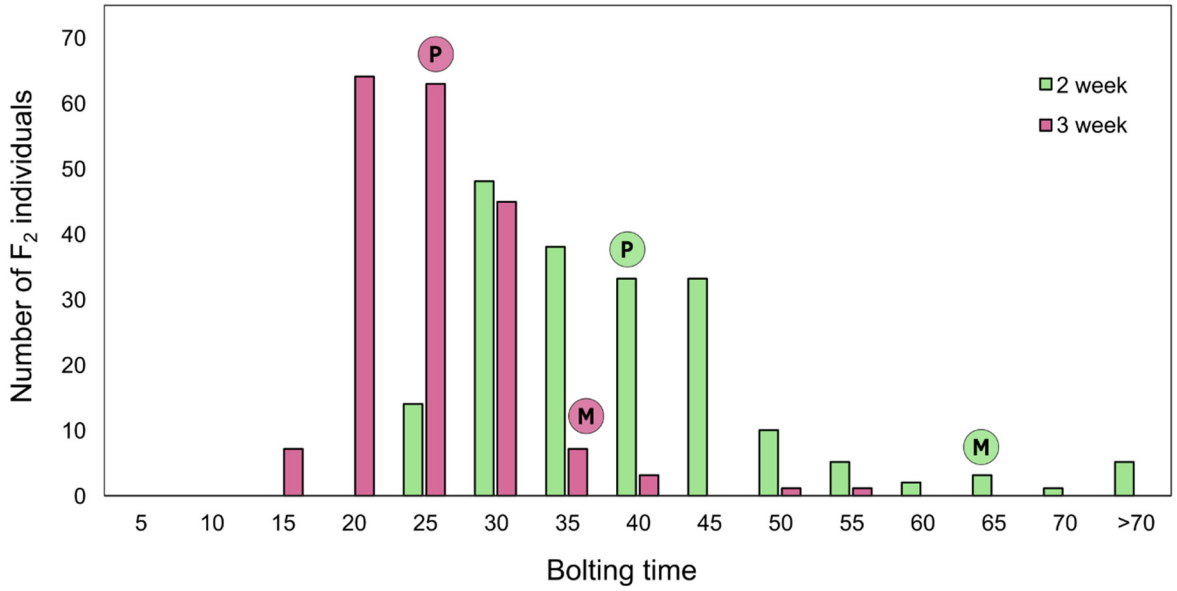

Figure 2. Bolting time of the F_2 population with two different vernalization periods (green, 2 weeks; pink, 3 weeks). M and P indicate the bolting times of the maternal line ('L432') and paternal line ('L285'), respectively.

QTLs controlling bolting time were identified via a high-density linkage map and phenotypic data from two F_2 populations. A total of 12 QTLs were detected from chromosomes 2, 3, 4, 5, 7, and 8 (Table 1; Figure 3). The genetic position is the 95% confidence interval of each QTL, and the physical position is the position of the linked bin markers. There were two QTLs from the 2-week vernalization treatment with relatively low LODs and PVEs compared with those from the 3-week treatment. RsBT7.1 and RsBT3.1 had the highest LOD and PVE scores, respectively. On chromosome 4, three QTLs were detected, and RsBT4.3 had a positive additive effect, indicating that the maternal genotype was associated with the late-bolting phenotype. No common QTLs from two different vernalization treatments or major QTL with large LOD values were identified. Therefore, we compared the QTL analysis results with GWAS results from radish germplasm.

Table 1. QTLs associated with bolting time in the F_2 population.

Vernalization Period	QTL Name	Chr.	Genetic Position (bp)	Physical Position (Mbp) *	LOD	PVE (%)	Additive Effect	Dominant Effect
2 weeks	*RsBT2.1*	2	260.1–268	36.3–38.6	3.3	0.1	1.9	4.9
	RsBT4.1	4	223.6–237.9	42.6–45.9	3.1	0.4	−3.6	−3.9
3 weeks	*RsBT2.2*	2	168.1–171.9	40.6–41.7	4.3	6.5	1.8	−0.3
	RsBT3.1	3	128.7–131.3	27.5–28.5	5.7	11.9	2.0	−1.3
	RsBT4.2	4	22–24.4	8.3–9.6	5.8	9.6	2.4	−0.2
	RsBT4.3	4	29.2–35.8	10.3–12.5	6.1	10.6	2.6	−0.3
	RsBT4.4	4	37.3–41.4	13.1–14.4	5.1	8.3	2.4	−0.1
	RsBT5.1	5	58–61.6	22.2–23.3	5.6	3.4	−2.1	−1.3
	RsBT7.1	7	14.7–16.3	4.7–5.2	7.7	5.1	−2.7	−1.5
	RsBT7.2	7	19.4–24.6	5.7–7.4	7.5	5.3	−2.8	−1.4
	RsBT8.1	8	52.3–53.7	14.2–16	4.1	5.5	−2.0	−0.2
	RsBT8.2	8	57.7–65.1	16.3–18.4	4.7	4.4	−2.0	−0.7

* Physical position was analyzed based on the 'Okute-Sakurajima' reference genome (v1.0). PVE, phenotypic variation explained by each QTL.

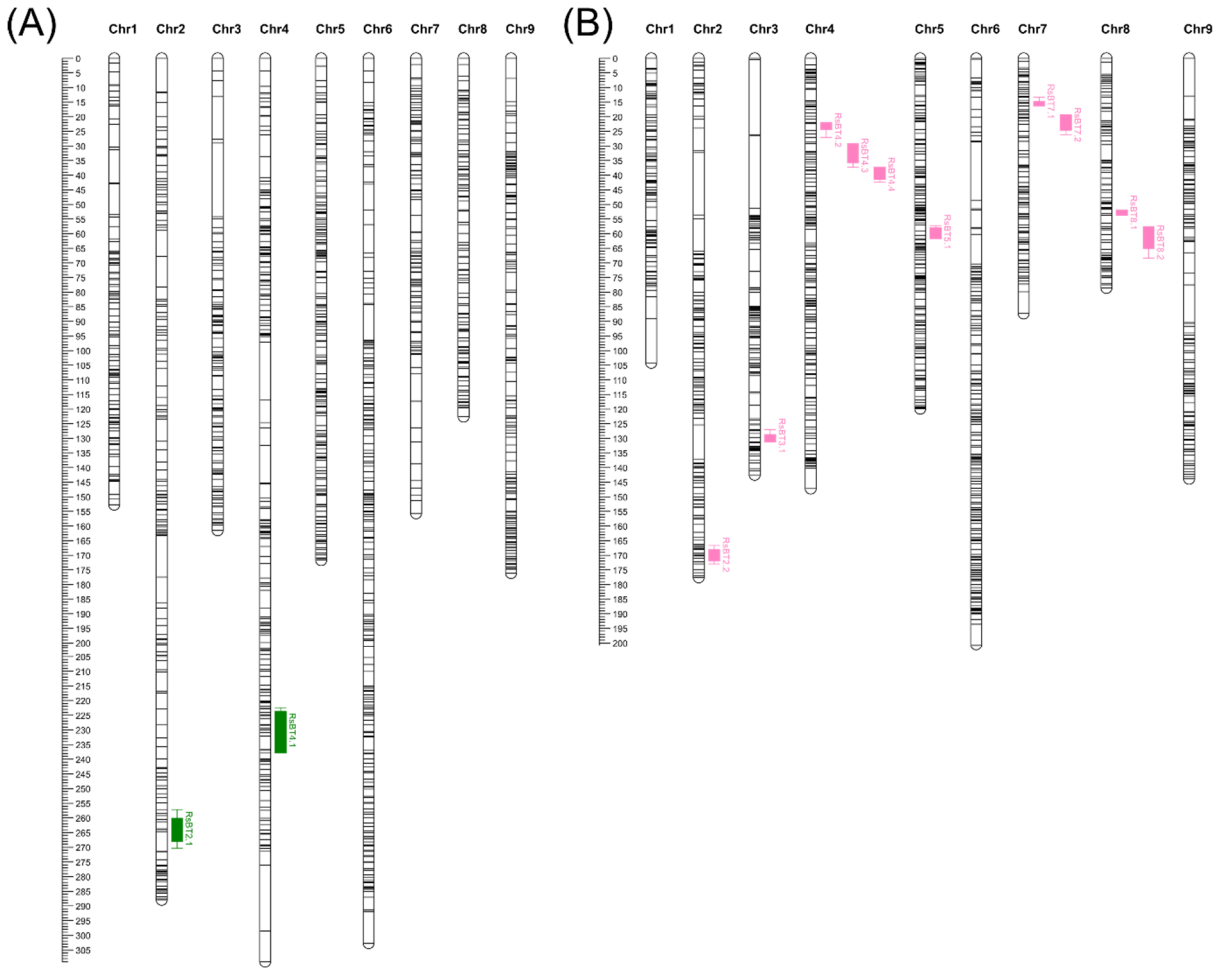

Figure 3. Genetic map and QTLs related to bolting time under cold treatment for 2 weeks (**A**) and 3 weeks (**B**). The QTL positions are indicated by boxes and bars, which represent 95% and 99% composite-invernal ranges, respectively.

3.3. GWAS for Bolting Time

Radish varieties that were evenly distributed on the phylogenetic tree were selected for GWAS from 173 varieties from Northeast Asian countries [41]. A total of 60 varieties were selected for the Korean, Chinese, and Japanese markets, including 28 late-bolting and 32 early-bolting varieties (Table S1). By resequencing, an average of 6.3 Gbp of reads were generated (Table S9), and a total of 74,852 SNPs were detected, with an average distance between SNPs of 4.7 kbp (Table S10). Using 3558 randomly selected SNPs, the population structure of the GWAS population was analyzed. It was grouped into three clusters (best K = 3, Figure 4A), and Clusters 1, 2, and 3 mostly consisted of Korean, Japanese, and Chinese varieties. Cluster 1 included only Korean early-bolting varieties, whereas Cluster 3 included Chinese early-bolting and Korean late-bolting varieties (Figure 4B). In Cluster 2, there were 18 Japanese, 8 Korean, and 10 Chinese varieties. The phylogenetic tree and population structure showed that East Asian varieties are genetically close, especially in white-skin radish market, which mostly clustered in Clusters 1 and 2. In Cluster 3, varieties for special markets were clustered, including radishes with green skin, green skin with red flesh, and red skin.

GWAS was conducted using 74,852 SNPs and binary phenotypes (early and late bolting). Using the BLINK method, seven significant SNPs associated with the bolting time of the GWAS population were identified (Table 2; Figure S2A). The phenotypic variation explained by each locus was between 3.26% and 39.68%. The most significant locus was R2_24501949, with a $-\log(P)$ value of 26.7, which can be a major locus for bolting time.

Association peaks were detected on R2_24501949 by GLM and MLM, but the −log(P) value did not exceed the threshold (Figure S2B,C). R2_36182766, R4_11119288, and R4_27162164 presented −log(P) values greater than 10.

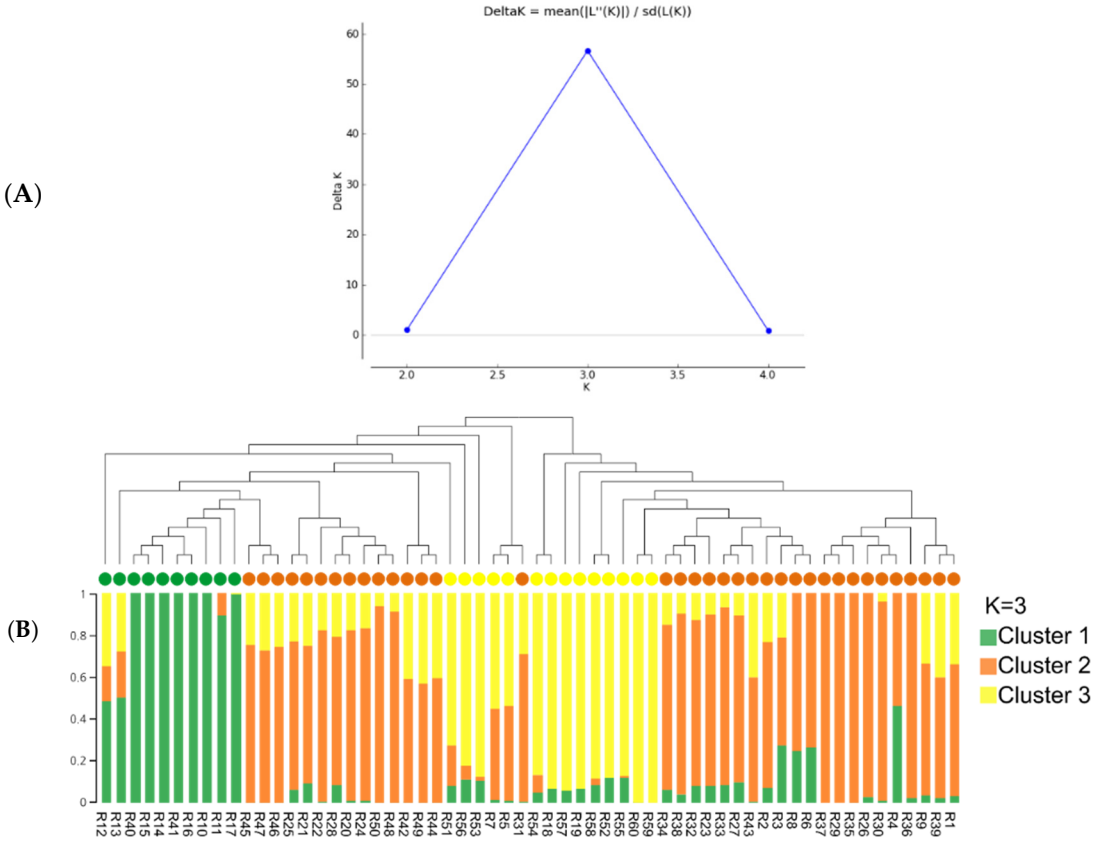

Figure 4. Genetic diversity of 60 radish cultivars. (**A**) The delta K distribution graph shows that the best K value is 3. (**B**) Dendrogram and population structure of cultivars.

Table 2. SNPs associated with bolting time detected by GWAS via the BLINK method.

SNP	Chr.	Position (bp)	−LOG(P)	MAF	PVE (%)
R2_24501949	2	24,501,949	26.7	0.32	39.68
R2_36182766	2	36,182,766	10.5	0.14	3.26
R3_19830045	3	19,830,045	8.8	0.18	10.24
R4_11119288	4	11,119,288	10.5	0.32	5.74
R4_27162164	4	27,162,164	10.1	0.05	15.13
R4_49690167	4	49,690,167	8.1	0.39	3.86
R9_28875894	9	28,875,894	7.6	0.07	5.67

MAF, minor allele frequency. PVE, phenotypic variation explained by each locus.

3.4. QTL–GWAS and Candidate Genes

Loci associated with bolting time detected via QTL and GWAS were compared. Two loci were colocalized on chromosomes 2 and 4. RsBT2.1 was located between 36.3 and 38.6 Mbp on chromosome 2, which is close to R2_36182766. RsBT4.3 and R4_11119288 were colocalized at 10.3–12.5 Mbp on chromosome 4 (Figure 5). This locus was expected to be a minor locus that can explain 0.1% and 3.3% of the phenotypic variation in the 'L432' × 'L285' F_2 and 60 GWAS populations, respectively. RsBT4.3 explained 10.6% of the total phenotypic variation in the F_2 population, and R4_11119288 explained 5.7% of the PVE in the GWAS population.

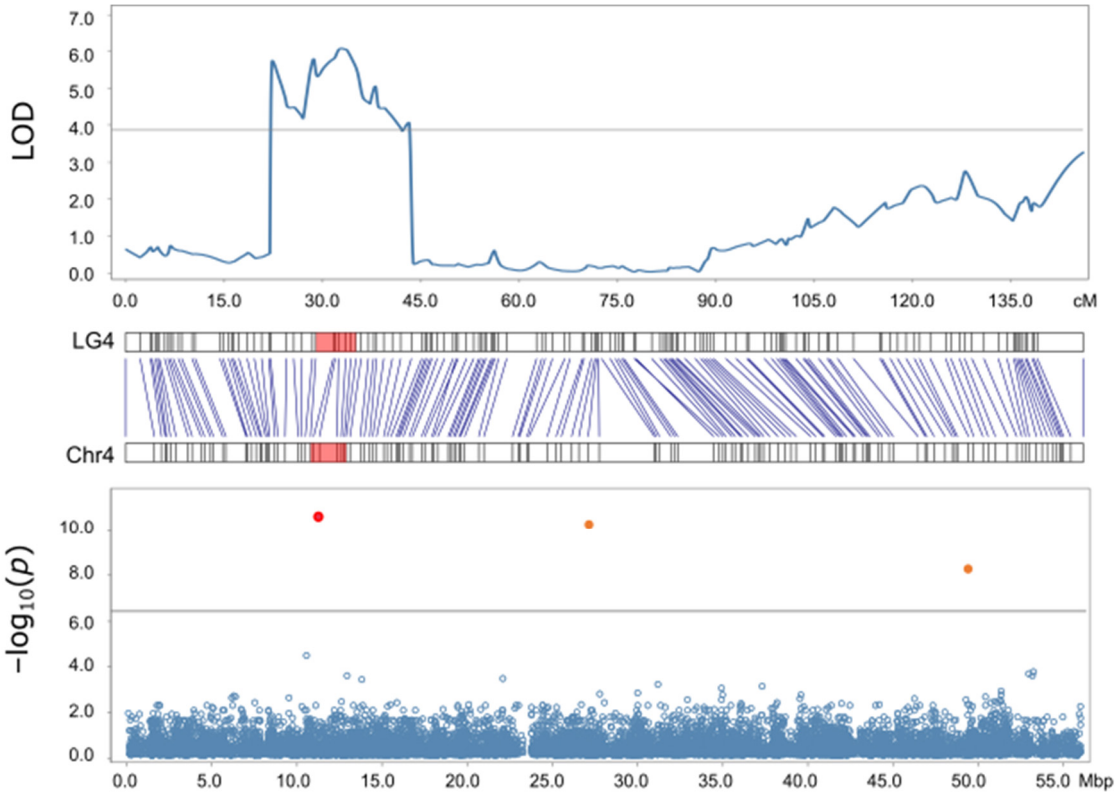

Figure 5. Comparison of the *RsBT4.3* and *R4_11119288* loci from the QTL analysis and GWAS, respectively. From the top, the LOD score of linkage Group 4, a comparison of the genetic and physical locations of bin markers, and a Manhattan plot of chromosome 4 are shown. Red shaded regions are common loci identified via QTL analysis and GWAS.

Candidate genes located at bolting time-related loci were identified via comparison with previously reported bolting- and vernalization-related genes [19,20,38–40]. A total of 14 candidate genes were detected (Table S11). The major locus R2_24501949 identified from GWAS was linked to RSAskr1.0R2g32110, the FLOWERING LOCUS C (FLC) homologous gene FLC2. Flowering-related genes, including CONSTANS-like (COL), APETALA (AP), FRIGIDA (FRI), and SQUAMOSA PROMOTER BINDING-LIKE (SPL), were located in the QTL regions. RSAskr1.0R2g34815, an ortholog of COL1, is located on RsBT2.1 and is 1 Mbp from R2_36182766. RSAskr1.0R4g44495, which is predicted to encode COL4, was linked to RsBT4.3 and R4_11119288.

3.5. Marker Development and Validation

To validate the effects of the QTL, two SNP-based HRM markers linked to RsBT4.3 and R4_11119288 were developed (Table S12). RsBT4.3_11.4 M and RsBT4.3_12.1 M markers were designed on the basis of SNPs that are polymorphic between 'L432' and 'L285'. These markers were also tested for another F_2 population derived from a cross between 'H16' (late-bolting inbred line) and 'red' (early-bolting germplasm). The 'H16' × 'red' F_2 population presented a wider range of bolting times than the 'L432' × 'L285' F_2 population did (Figure S3). RsBT4.3_11.4 M was polymorphic for both F_2 populations, whereas RsBT4.3_12.1 M could be applied to only the 'L432' × 'L285' F_2 population. The RsBT4.3_11.4 M marker presented a clear melting peak pattern for the maternal, paternal and heterozygous genotypes (Figure 6A). The bolting time of F_2 individuals with the late-bolting genotype was significantly different from that with the early-bolting genotype (Figure 6B,C; Table S13). The heterozygous individuals in the two populations presented inconsistent phenotypes.

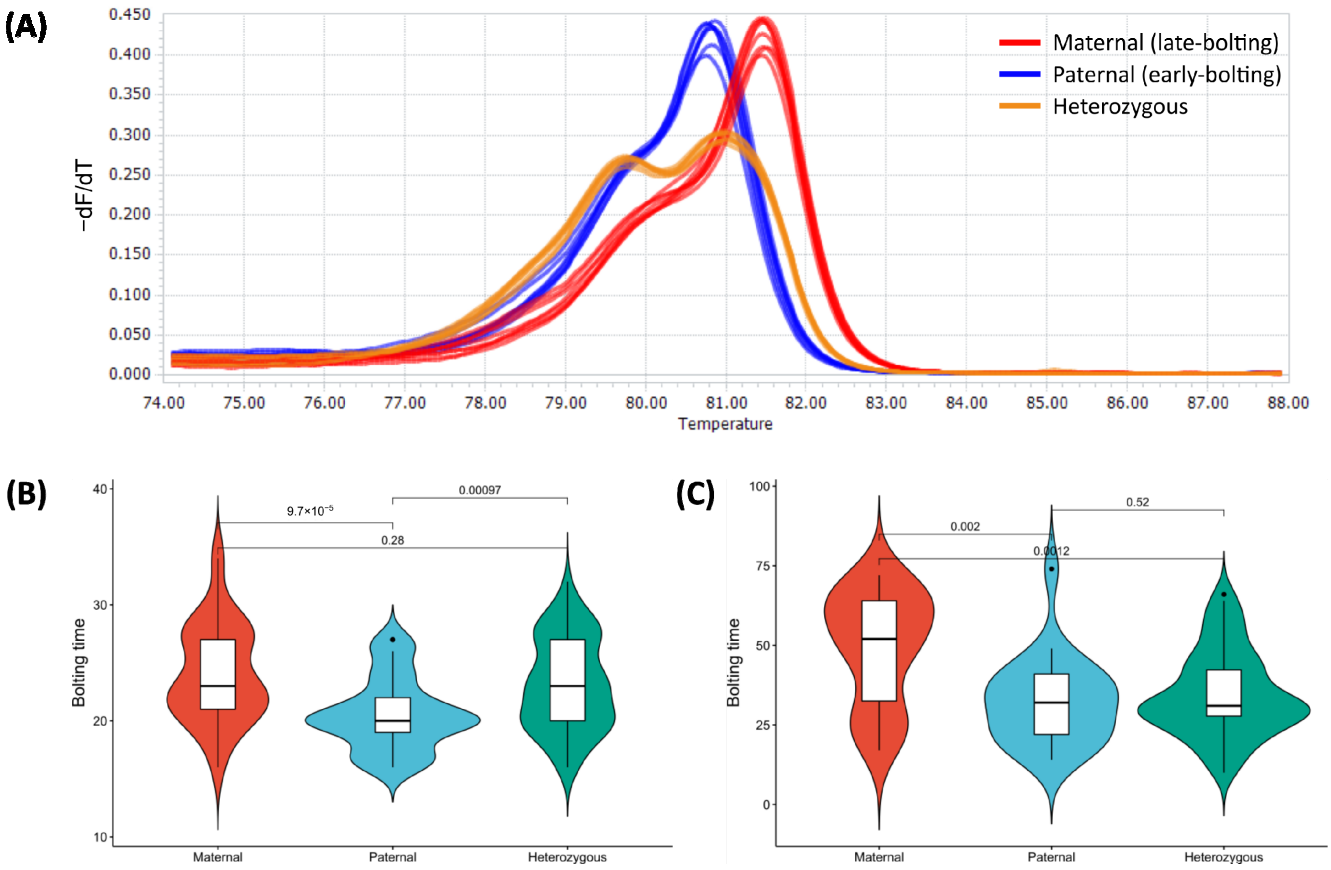

Figure 6. Development of an HRM marker linked to RsBT4.3. (**A**) Normalized melting peaks of the 'RsBT4.3_11.4 M' marker. Violin plots showing bolting time by marker genotype of (**B**) 'L432' × 'L285' 175 F2 and (**C**) 'H16' × 'red' 93 F2 individuals.

4. Discussion

Bolting in radishes physiologically depletes the nutrients in the plant, as all resources are directed toward seed formation. This significantly reduces both the quantity and quality of the radish root as a marketable product, which is why producers prefer varieties with delayed flowering to ensure stable quality. For the analysis of bolting formation, we used radishes treated with vernalization for two and three weeks. The results revealed more QTLs identified after the three-week treatment, suggesting that the three-week vernalization treatment was more effective than the two-week vernalization treatment at comparing the states of the two parental lines. As shown in Figure 2, the difference in the bolting formation period between the parental lines was more pronounced after the two-week treatment than after the three-week treatment. Although further experiments are needed to understand the effects of vernalization duration on bolting formation, as shown in Figure S1, we observed that the longer the vernalization treatment was, the shorter the time required for bolting formation. These findings suggest that adequate temporal maturity is necessary for bolting formation.

We identified 12 QTLs related to bolting in our experiment, as shown in Table 1. Using methods such as BLINK, we analyzed the GWAS results and identified 14 candidate genes, as shown in Tables 2 and S11. These results were compared with those of previously reported QTL and RNA-Seq studies, and we identified *FLC2* and *COL4* as potential candidates. The *FLC* gene is expected to be a major gene distinguishing between late and early bolting, as indicated by previous studies. Three *FLC* genes (*RsFLC1*, *RsFLC2*, and *RsFLC3*) in radishes have been identified through genome sequencing [42,43], transcriptome analysis [44], and QTL analysis [19]. The overexpression of each of the three radish *FLC* (*RsFLC*) genes delayed flowering in Arabidopsis, demonstrating that each *RsFLC* functions

as a flowering repressor. Recently, a candidate gene and allelic variation responsible for the extremely late bolting trait in radishes were identified. F_2 populations were created by crossing early- and late-bolting varieties, and the regulatory roles and interactions between the insertion/deletion alleles of *RsFLC1* and *RsFLC2* were evaluated [18].

However, this single gene alone cannot fully explain the bolting phenotype. Therefore, we focused on the *COL4* gene as a possible candidate. The three main flowering pathways, gibberellin, vernalization, and photoperiod, are involved in regulating flowering integrators. In the photoperiod pathway of radishes, *CO* is suppressed by *RsLHY*, *RsGI*, and *RsTEM1* (*RsTEMPRANILLO1*). *TEM1* negatively regulates GA biosynthesis and *CO* while repressing the transcription of *FT* and *SOC1*. The expression changes in *RsVRN1* in radishes suggest that it can significantly induce the transition to flowering under vernalization conditions. Each flowering pathway is either independent or interconnected, ultimately converging on flowering integrators such as *FT*, *SOC1*, and *LFY* [21,38]. Among the flowering-related genes, the *COL* gene plays a crucial role in the photoperiod pathway, and it was identified as a significant QTL in this study.

Based on QTL-GWAS analysis, we developed bolting time-associated markers (Table S12; Figure 6). The bolting time of F_2 individuals with the maternal genotype of the RsBT4.3_11.4 M marker was longer than that of F_2 individuals with the paternal genotype in the 'L432' × 'L285' and 'H16' × 'red' F_2 populations. However, the heterozygous genotyped individuals showed relatively late and early bolting in the two populations. Additional studies, such as GWASs using large germplasm sets with quantitative bolting time values, can improve the accuracy of predicting bolting time or selecting late-bolting individuals. This paper focused on exploring the genetic factors that control traits related to delayed bolting within the early bolting market. Additionally, major candidate genes controlling late and early bolting were proposed. Marker-assisted integration of candidate genes could be utilized to develop late-bolting varieties.

5. Conclusions

In this study, we investigated the genetic factors controlling bolting time in radishes, focusing on F_2 populations treated with vernalization for two and three weeks. Our findings revealed that bolting time is a quantitative trait influenced by multiple QTLs, with significant variation observed between the parental lines and F_2 populations. We identified 12 QTLs associated with bolting time. Through GWAS analysis of 60 radish varieties, we identified seven loci, and these loci were compared with QTLs on the basis of the physical position of the reference genome. Through QTL and GWAS analyses, 14 candidate genes related to the regulation of bolting and flowering time, including the major genes *FLC2* and *COL4*, were proposed. Our study confirmed that *FLC2* is a key gene involved in differentiating late and early bolting, which is consistent with the findings of previous studies. In addition, *COL4* emerged as another important gene for controlling bolting time in early-bolting-type radishes. The development of SNP-based markers linked to *RsBT4.3* and *R4_11119288*, where *COL4* is located, provided significant insights into the genetic mechanisms controlling bolting. This research emphasizes the complexity of breeding for delayed bolting in radishes, particularly in the context of the East Asian market. While significant progress has been made in identifying and validating genetic markers associated with late bolting, the integration of multiple genes will be essential for developing stable late-bolting radish lines in the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112700/s1, Figure S1: Bolting time of parental lines with four different vernalization treatments; Figure S2: Manhattan plot of GWAS for bolting time; Figure S3: Distribution of bolting time in the 'H16' × 'red' F_2 population after three weeks of vernalization treatment; Table S1: Radish germplasm genotyped for GWAS; Table S2: Bolting time of 'L432' × 'L285' F_2 plants after 2 weeks of vernalization treatment; Table S3: Bolting time of 'L432' × 'L285' F_2 plants after 3 weeks of vernalization treatment; Table S4: Linkage map of F_2 populations after 2 and 3 weeks of vernalization treatment; Table S5: Genotype of SNPs detected from 'L432' × 'L285' F2 plants

after 2 weeks of vernalization treatment; Table S6: Genotype of SNPs detected from 'L432' × 'L285' F2 plants after 3 weeks of vernalization treatment; Table S7: Genotype and genetic position of bin markers of 'L432' × 'L285' F2 plants after 2 weeks of vernalization treatment; Table S8: Genotype and genetic position of bin markers of 'L432' × 'L285' F2 plants after 3 weeks of vernalization treatment; Table S9: Summary of resequencing data for 60 radish germplasms used for GWAS; Table S10: Summary of significant SNPs used for GWAS; Table S11: Candidate genes located at loci associated with bolting time; Table S12: Primer sequences for HRM analysis; Table S13: HRM analysis results of the 'L432' × 'L285' and 'H16' × 'red' F_2 populations; Table S14: SNPs located in FLC2 and COL4, detected from GWAS population.

Author Contributions: Conceptualization, K.H., Y.-R.L., E.-S.L., C.-S.J. and D.-S.K.; Methodology, K.H., H.-I.A., H.-B.Y. and J.L.; Formal analysis, H.-I.A.; Investigation, K.H., H.-B.Y., Y.-R.L. and E.-S.L.; Resources, J.L. and C.-S.J.; Writing—original draft, K.H. and H.-I.A.; Supervision, D.-S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Research Project and 2023 the RDA Fellowship Program of National Institute of Horticultural and Herbal Science, Rural Development Administration, Republic of Korea (Project No: PJ01504002).

Data Availability Statement: The data are contained within the article and the Supplementary Materials; further inquiries may be directed to the corresponding author. Sequencing data of GWAS population and F_2 population have been deposited in the National Agricultural Biotechnology Information Center (https://nabic.rda.go.kr, accession number NN-9168 and NN-9169, accessed on 16 October 2024).

Acknowledgments: We would like to thank Dayi International Seed for providing us with East Asian radish varieties of seeds.

Conflicts of Interest: Author Chang Soon Jang is employed by the company Hankook Seed Co. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

1. Amasino, R.M.; Michaels, S.D. The Timing of Flowering. *Plant Physiol.* **2010**, *154*, 516–520. [CrossRef] [PubMed]
2. Srikanth, A.; Schmid, M. Regulation of flowering time: All roads lead to Rome. *Cell Mol. Life Sci.* **2011**, *68*, 2013–2037. [CrossRef] [PubMed]
3. Chouard, P. Vernalizaion and its Relation to Dormancy. *Annu. Rev. Plant Physiol.* **1960**, *11*, 191–238. [CrossRef]
4. Bernier, G.; Havelange, A.; Houssa, C.; Petitjean, A.; Lejeune, P. Physiological Signals That Induce Flowering. *Plant Cell* **1993**, *5*, 1147–1155. [CrossRef] [PubMed]
5. Reeves, P.H.; Coupland, G. Response of plant development to environment: Control of flowering by daylength and temperature. *Curr. Opin. Plant Biol.* **2000**, *3*, 37–42. [CrossRef]
6. Engelen-Eigles, G.; Erwin, J.E. A model plant for vernalization studies. *Sci. Hortic.* **1997**, *70*, 197–202. [CrossRef]
7. Michaels, S.D.; Amasino, R.M. FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* **1999**, *11*, 949–956. [CrossRef]
8. De Lucia, F.; Crevillen, P.; Jones, A.M.; Greb, T.; Dean, C. A PHD-polycomb repressive complex 2 triggers the epigenetic silencing of FLC during vernalization. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 16831–16836. [CrossRef]
9. Searle, I.; He, Y.; Turck, F.; Vincent, C.; Fornara, F.; Kröber, S.; Amasino, R.A.; Coupland, G. The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. *Genes Dev.* **2006**, *20*, 898–912. [CrossRef]
10. Bastow, R.; Mylne, J.S.; Lister, C.; Lippman, Z.; Martienssen, R.A.; Dean, C. Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature* **2004**, *427*, 164–167. [CrossRef]
11. Michaels, S.D.; Himelblau, E.; Kim, S.Y.; Schomburg, F.M.; Amasino, R.M. Integration of Flowering Signals in Winter-Annual Arabidopsis. *Plant Physiol.* **2005**, *137*, 149–156. [CrossRef] [PubMed]
12. Parcy, F. Flowering: A time for integration. *Int. J. Dev. Biol.* **2005**, *49*, 585–593. [CrossRef] [PubMed]
13. Yanovsky, M.J.; Kay, S.A. Molecular basis of seasonal time measurement in Arabidopsis. *Nature* **2002**, *419*, 308–312. [CrossRef] [PubMed]
14. Amasino, R. Vernalization, Competence, and the Epigenetic Memory of Winter. *Plant Cell* **2004**, *16*, 2553–2559. [CrossRef]
15. Amasino, R. Seasonal and developmental timing of flowering. *Plant J.* **2010**, *61*, 1001–1013. [CrossRef]
16. Sung, S.; Schmitz, R.J.; Amasino, R.M. A PHD finger protein involved in both the vernalization and photoperiod pathways in Arabidopsis. *Genes Dev.* **2006**, *20*, 3244–3248. [CrossRef]

17. Finnegan, E.J.; Dennis, E.S. Vernalization-Induced Trimethylation of Histone H3 Lysine 27 at FLC Is Not Maintained in Mitotically Quiescent Cells. *Curr. Biol.* **2007**, *17*, 1978–1983. [CrossRef]
18. Mitsui, Y.; Yokoyama, H.; Nakaegawa, W.; Tanaka, K.; Komatsu, K.; Koizuka, N.; Okuzaki, A.; Matsumoto, T.; Takahara, M.; Tabei, Y. Epistatic interactions among multiple copies of FLC genes with naturally occurring insertions correlate with flowering time variation in radish. *AoB Plants* **2023**, *15*, plac066. [CrossRef]
19. Wang, Q.; Zhang, Y.; Zhang, L. A naturally occurring insertion in the RsFLC2 gene associated with late-bolting trait in radish (*Raphanus sativus* L.). *Mol. Breed.* **2018**, *38*, 137. [CrossRef]
20. Hu, T.; Wei, Q.; Wang, W.; Hu, H.; Mao, W.; Zhu, Q.; Bao, C. Genome-wide identification and characterization of CONSTANS-like gene family in radish (*Raphanus sativus*). *PLoS ONE* **2018**, *13*, e0204137. [CrossRef]
21. Jung, H.; Jo, S.H.; Jung, W.Y.; Park, H.J.; Lee, A.; Moon, J.S.; Seong, S.Y.; Kim, J.-K.; Kim, Y.-S.; Cho, H.S. Gibberellin Promotes Bolting and Flowering via the Floral Integrators RsFT and RsSOC1-1 under Marginal Vernalization in Radish. *Plants* **2020**, *9*, 594. [CrossRef] [PubMed]
22. Jin, Y.; Luo, X.; Li, Y.; Peng, X.; Wu, L.; Yang, G.; Xu, X.; Pei, Y.; Li, W.; Zhang, W. Fine mapping and analysis of candidate genes for qBT2 and qBT7.2 locus controlling bolting time in radish (*Raphanus sativus* L.). *Theor. Appl. Genet.* **2023**, *137*, 445. [CrossRef] [PubMed]
23. Ma, Y.; Chhapekar, S.S.; Rameneni, J.J.; Kim, S.; Gan, T.H.; Choi, S.R.; Lim, Y.P. Identification of QTLs and Candidate Genes Related to Flower Traits and Bolting Time in Radish (*Raphanus sativus* L.). *Agronomy* **2021**, *11*, 1623. [CrossRef]
24. Gan, C.X.; Cui, L.; Pang, W.X.; Wang, A.H.; Yu, X.Q.; Deng, X.H.; Song, L.P.; Piao, Z.Y. QTL Mapping of Bolting and Flowering Traits Based on High Density Genetic Map of Radish. *Acta Hortic. Sin.* **2021**, *48*, 1273–1281.
25. Ahn, H.-I.; Han, K.; Yang, H.-B.; Lee, E.S.; Lee, Y.-R.; Kim, J.; Park, H.Y.; Kim, D.-S. Development and Investigation of HRM Markers to Discriminate Two Ogura Cytoplasmic Male Sterility Restorer Genes in Radish. *Agronomy* **2023**, *14*, 43. [CrossRef]
26. Elshire, R.J.; Glaubitz, J.C.; Sun, Q.; Poland, J.A.; Kawamoto, K.; Buckler, E.S.; Mitchell, S.E. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS ONE* **2011**, *6*, e19379. [CrossRef] [PubMed]
27. Andrews, A.; Bobo, L. Performance Measurement and Assessment using Dartfish Software. In *E-Learn: World Conference on E-Learning in Corporate, Government, Healthcare, and Higher Education 2010*; Sanchez, J., Zhang, K., Eds.; Association for the Advancement of Computing in Education (AACE): Orlando, FL, USA, 2010; pp. 407–408.
28. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef] [PubMed]
29. Shirasawa, K.; Hirakawa, H.; Fukino, N.; Kitashiba, H.; Isobe, S. Genome sequence and analysis of a Japanese radish (*Raphanus sativus*) cultivar named 'Sakurajima Daikon' possessing giant root. *DNA Res.* **2020**, *27*, dsaa010. [CrossRef]
30. Vasimuddin, M.; Misra, S.; Li, H.; Aluru, S. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. In Proceedings of the 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS), Rio de Janeiro, Brazil, 20–24 May 2019; pp. 314–324.
31. Van der Auwera, G.A.; O'Connor, B.D. *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra*, 1st ed.; O'Reilly Media: Newton, MA, USA, 2020.
32. Han, K.; Jeong, H.J.; Yang, H.B.; Kang, S.M.; Kwon, J.K.; Kim, S.; Choi, D.; Kang, B.-C. An ultra-high-density bin map facilitates high-throughput QTL mapping of horticultural traits in pepper (*Capsicum annuum*). *DNA Res.* **2016**, *23*, 81–91. [CrossRef]
33. De Givry, S.; Bouchez, M.; Chabrier, P.; Milan, D.; Schiex, T. CARHTA GENE: Multipopulation integrated genetic and radiation hybrid mapping. *Bioinformatics* **2005**, *21*, 1703–1704. [CrossRef]
34. Wang, S.; Basten, C.J.; Zeng, Z.-B. Windows QTL Cartographer 2.5. 2012. Available online: http://statgen.ncsu.edu/qtlcart/WQTLCart.htm (accessed on 1 August 2012).
35. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
36. Kim, M.; Nguyen, T.T.P.; Ahn, J.-H.; Kim, G.-J.; Sim, S.-C. Genome-wide association study identifies QTL for eight fruit traits in cultivated tomato (*Solanum lycopersicum* L.). *Hortic. Res.* **2021**, *8*, 203. [CrossRef] [PubMed]
37. Huang, M.; Liu, X.; Zhou, Y.; Summers, R.M.; Zhang, Z. BLINK: A package for the next level of genome-wide association studies with both individuals and markers in the millions. *GigaScience* **2018**, *8*, giy154. [CrossRef]
38. Jung, W.Y.; Park, H.J.; Lee, A.; Lee, S.S.; Kim, Y.-S.; Cho, H.S. Identification of Flowering-Related Genes Responsible for Differences in Bolting Time between Two Radish Inbred Lines. *Front. Plant Sci.* **2016**, *7*, 1844. [CrossRef]
39. Nie, S.; Li, C.; Xu, L.; Wang, Y.; Huang, D.; Muleke, E.M.; Sun, X.; Xie, Y.; Liu, L. De novo transcriptome analysis in radish (*Raphanus sativus* L.) and identification of critical genes involved in bolting and flowering. *BMC Genom.* **2016**, *17*, 389. [CrossRef] [PubMed]
40. Li, Y.; Luo, X.; Peng, X.; Jin, Y.; Tan, H.; Wu, L.; Li, J.; Pei, Y.; Xu, X.; Zhang, W. Development of SNP and InDel markers by genome resequencing and transcriptome sequencing in radish (*Raphanus sativus* L.). *BMC Genom.* **2023**, *24*, 445. [CrossRef]
41. Han, K.; Yang, H.-B.; Lee, J.; Lee, E.S.; Lee, H.-E.; Kim, D.-S. Selection of the Northeast Asian radish varieties for the development of the cost-cutting and efficient marker assisted backcrossing system. *Horticultural Science and Technology.* **2022**, *40* (Suppl. 2), 289.
42. Kitashiba, H.; Li, F.; Hirakawa, H.; Kawanabe, T.; Zou, Z.; Hasegawa, Y.; Tonosaki, K.; Shirasawa, S.; Fukushima, A.; Yokoi, S.; et al. Draft Sequences of the Radish (*Raphanus sativus* L.) Genome. *DNA Res.* **2014**, *21*, 481–490. [CrossRef]

43. Jeong, Y.-M.; Kim, N.; Ahn, B.O.; Oh, M.; Chung, W.-H.; Chung, H.; Jeong, S.; Lim, K.-B.; Hwang, Y.-J.; Kim, G.-B.; et al. Elucidating the triplicated ancestral genome structure of radish based on chromosome-level comparison with the Brassica genomes. *Theor. Appl. Genet.* **2016**, *129*, 1357–1372. [CrossRef]
44. Yi, G.; Park, H.; Kim, J.-S.; Chae, W.B.; Park, S.; Huh, J.H. Identification of three FLOWERING LOCUS C genes responsible for vernalization response in radish (*Raphanus sativus* L.). *Hortic. Environ. Biotechnol.* **2015**, *55*, 548–556. [CrossRef]