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Genetic Map Construction, QTL Mapping, and Candidate Genes Screening of Grain Size Traits in Common Buckwheat (*Fagopyrum esculentum* M.)

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Abstract: Common buckwheat (*Fagopyrum esculentum* M.) is an important pseudo-cereal crop and contains an abundance of nutrients and bioactive compounds. However, the yield of buckwheat is low compared to that of other major crops. QTL mapping and candidate gene screening of grain size are very important for increasing production in common buckwheat through molecular breeding in the future. In the present study, an F_1 segregating population with 217 individuals was established using a cross between Ukraine daliqiao (UD) and Youqiao 2 (YQ2) that showed a significant difference in grain size. The InDel and SSR primers were developed based on transcriptome sequencing between parents in the previous study. We constructed a genetic linkage map, including 39 SSR loci and 93 InDel loci, with a total length of 1398.33 cM and an average spacing of 10.59 cM. Combined with the grain size phenotype data of the F_1 population, a total of 14 QTL were detected, including 6-grain length QTL, 3-grain width QTL, and 5 hundred-grain-weight QTL. QTL of grain width and hundred-grain weight were all detected near SWU_Fe_InDel086 and SWU_Fe_InDel076. Some putative candidate genes with the ex1pression or InDel difference between parents were screened within the QTL interval. This study would lay the foundation for map-based cloning and molecular mechanism of grain size and ultimately improvement of yield in common buckwheat.

Keywords: common buckwheat; genetic map; QTL-mapping; candidate genes; grain size

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1. Introduction

Common Buckwheat (*Fagopyrum esculentum* Moench; 2n = 2x = 16), the family Polygonaceae, genus Fagopyrum, was an annual crop and widely cultivated in temperate zones. As a minor and pseudo-cereal, common buckwheat had the characteristics of self-incompatibility, short growth period, strong adaptability, and high resistance to various environmental stressors [1]. Furthermore, buckwheat is also important in supporting the dietary health of humans [2,3] for containing high levels of flavonoids, protein, starch, and dietary fiber in the grain [4] and special nutrients that are not produced by major cereal crops, such as phytochemicals, soluble fiber, and rutin [5,6]. As a result, common buckwheat is currently attracting breeders and the attention of various stakeholders. However, common buckwheat has lower yields than the major cereals and shorter history of modern breeding. Therefore, it is necessary to establish efficient breeding programs for common buckwheat to increase grain production.

However, owing to heteromorphic self-incompatibility controlled by the S-locus [7,8], the breeding of common buckwheat is difficult. The efficiency of mass selection is lower, and the inbreeding depression seriously hinders genetic improvement [9,10]. Great advances in DNA marker technologies have provided new insights for improving common

buckwheat. Based on the high-density molecular marker genetic linkage maps, quantitative trait loci (QTL) of yield and quality traits can be mapped, and the molecular markers tightly linked to the traits were selected for screening the genotypes for target traits. Therefore, combining conventional breeding methods and marker-assisted breeding will provide an effective approach to developing common buckwheat new cultivars with improved yield and quality.

Recently, various genetic molecular marker systems have been developed on common buckwheat, such as the RAPD marker [11–14], amplified fragment length polymorphism (AFLP) markers [15–17], SSR marker [18–22], expressed sequence tag (EST) markers [23], and SNP markers based on Next Generation Sequencing technology [24,25]. However, the number of molecular markers was fairly limited and did not span the entire genome due to the backward genetic study of common buckwheat.

The allogamous reproductive system of common buckwheat works as a barrier, which has hindered the construction of a suitable mapping population, such as the NIL, RIL, or NAM population. The heterozygous parents with relatively distant relatives were used for constructing the population mapping, which caused many isolated loci in the F_1 population. The polymorphic loci in parents and 1:1 separation in F_1 were selected to simulate the test crossing sites in inbred lines for plotting. This drawing method was called the pseudo-testcross strategy [26]. The method has been successfully applied to a variety of outcrossing plants, such as switchgrass [27], butterfly orchid [28], tea tree [29], sugarcane [30], apple [31], sage [32]. The pseudo-testcross strategy provided an effective method for constructing a genetic linkage map in common buckwheat. Based on microsatellite and AFLP markers, a linkage map of common buckwheat was constructed using a pseudo-testcross mapping strategy for 80 F_1 individuals [19]. Likewise, by using the pseudo-testcross strategy, a high-density linkage map based on the array-based genotyping system was constructed with 178 F_1 progeny, which was composed of 756 loci and included 8884 markers [24]. The above studies confirmed the usefulness of the pseudo-testcross mapping strategy for constructing the linkage maps, even for allogamous populations.

Currently, only a few studies about QTL mapping for agronomically important traits of common buckwheat have been reported. Yabe et al. [24] constructed a genome-wide linkage map with array-based markers (including 8884 markers, spanning 756 loci) onto eight linkage groups. They identified a QTL controlling main stem length, which had been successfully applied to genomic selection to increase the yield of common buckwheat [33]. In addition, to circumvent the complex genetic patterns caused by the cross-pollinating reproductive system, an F_4 population derived from a cross between autogamous lines were used for QTL mapping controlling the photoperiod sensitivity of common buckwheat [23]. Three ear sprouting resistance buckwheat lines (KY29, KY28, and NF1) and a buckwheat self-compatible line KSC7 with easy ear sprouting were used to construct F_2 populations, and three genetic linkage maps were constructed by NGS-BSA high-throughput sequencing technology to identify two major and two minor QTL for ear sprout [34,35]. Such studies suggested that QTL analysis will contribute to allogamous crop breeding via conventional MAS for major genes. In general, the genetic map of common buckwheat is small, and most of them have low density. The number of QTL is small, and the distance is far from their linkage markers, which cannot meet the requirements of molecular marker-assisted selection and map cloning.

Grain size is an important factor affecting grain yield. Big grain has always been one of the main goals of breeders. However, studies on the grain size of common buckwheat are rarely reported. In the early stage of the project, transcriptome sequencing was performed on the 5 and 10 days post-anthesis (DPA) grains of large grain buckwheat variety Ukraine daliqiao (UD) and small grain buckwheat variety Youqiao 2 (YQ2), and the different-expressed genes related to grain size were screened [36]. In this study, we selected UD and YQ2 as mapping parents and constructed a genetic linkage map using SSR markers and InDel markers to screen the QTL and candidate genes affecting grain size-related traits,

which would lay the foundation for map-based cloning and the molecular basis of grain size and ultimately utilization for improvement of yield in common buckwheat.

2. Materials and Methods

2.1. Mapping Population and Grain Size Traits Evaluation

In this study, two common buckwheat varieties with grain size differences, Ukraine daliqiao (UD) and Youqiao 2 (YQ2), were used to produce the segregating population. The two parents were crossed in the winter of 2018 at Southwest University, Chongqing, China. An F_1 population of 217 individuals in the mapping population and the two parental lines was planted in the autumn of 2019. All individuals from the F_1 population and their parents were hand-harvested when 70–80% of total seeds changed their color from green to black. Seeds were air-dried for two weeks before measurement. Grain length, grain width, hundred-grain weight, and the ratio of length-width of each individual were measured.

2.2. Primer Development and Marker Validation

A total of 320 pairs of SSR primers and 336 pairs of InDel primers were employed in the present study. They were synthesized by Beijing Genomics Institute Co., Ltd. (Beijing, China) to construct a genetic map and QTL mapping. Among these primers, 224 SSR primers pairs and 24 InDel primers pairs were designed in our laboratory [37]. The other 100 SSR and 312 InDel primers were designed in this present study (Table S1) based on the transcriptome sequencing data, which was carried out on 5 and 10 days on grains of parents UD and YQ2, respectively (Fang et al., 2019, accession: PRJNA523295). In this study, MISA (MIcroSAtellite; http://pgrc.ipk-gatersleben.de/misa, 1 May 2019) and SAM tools [38] were employed for SSR mining and identification based on transcriptome data. The minimum number of repeats used to select the SSRs was ten for mono-nucleotide repeats, six for di-nucleotide repeats, five for tri-nucleotide repeats, and three for tetra-, penta-, and hexanucleotide repeats. SSR and InDel primers were designed by Premier 5.0 (PREMIER Biosoft International, Palo Alto, CA, USA) with the following criteria: GC content of 40–60%, primer lengths of 18–23 bases, an annealing temperature of 40–60 °C, and PCR product size of 100–300 bp.

Genomic DNA samples of the two parents and 217 F_1 progeny individuals were extracted from young leaves according to the modified CTAB method [39]. All these primer pairs were first screened between two parents for polymorphism. The primer pairs, which showed clear polymorphism, were used to detect the genotype of F_1 population individuals. PCR amplification and product test were performed as described by Zhang et al. [39]. Clear polymorphic DNA bands on the gels were used for genotyping. Loci were named with the primer name. For multiple polymorphic loci revealed by the same primer, an extra letter was added, followed by the primer name, such as a/b/c, indicating the molecular size from the smallest to the largest.

2.3. Genetic Map Construction and QTL Analysis

JoinMap 4.0 [40] was used for linkage analysis and map construction. As separate data sets, the linked loci or small groups were then recalculated to construct a new draft map. To avoid any possible errors, the positions or orders of some loci were suspicious, and gels of these loci were rescored, even re-run. Loci that could not be anchored to any linkage group were discarded. Map distances were calculated using Kosambi's mapping function.

The multiple QTL mapping was performed by MapQTL 6.0 [41] to detect putative QTL and estimate their effects. The LOD threshold of significant QTL was calculated by 1000 permutation tests with a significance level of P at 0.05. The QTL with the LOD value > 2.5 were declared as putative QTL in the present study. Additive effects were defined concerning the alleles of UD. Therefore, the positive genetic effect of each QTL indicated that the allele of UD increased the phenotypic value. In contrast, the negative effect indicated that the allele of YQ2 increased the phenotypic value. QTL name was started with 'q', followed by a trait abbreviation (GW for grain width, GL for grain length, HGW

for hundred-kernel-weight), linkage group number, and the number of QTL controlling the same trait on the same linkage group. The graphic representation of linkage groups and QTL bars was created using Map Chart 2.2 [42].

2.4. Validation of Candidate Genes by RNA-Seq

The RNA-Seq data was from our previous report [36]. RNA Samples were collected at 5 and 10 DPA grains of parents UD and YQ2 for cDNA library construction, respectively. Two replicates were used for each sample. The libraries were sequenced on an Illumina Hiseq platform, and paired-end reads were generated by Novogene Bioinformatics Technology Co., Ltd., Beijing, China (www.novogene.cn, 1 May 2019). Gene expression levels were estimated by RSEM for each sample. Differential expression analysis of two samples was performed using the DEGseq (2010) R package. In the present study, based on marker information in QTL location interval, Combined the differential expression analysis and NR annotation information of unigenes corresponding to the markers, the candidate genes related to the grain size of common buckwheat were screened and analyzed.

3. Results

3.1. The Phenotypic Data Analysis of Parents and F_1 Population

The phenotype comparison of UD and YQ2 showed that the grain length, grain width, and grain weight of UD were significantly greater than that of YQ2, while the length-width ratio was slightly less than that of YQ2 (Figure 1). Compared with YQ2, UD showed a grain length that was increased by 35.47%, a grain width increased by 52.99%, and hundred-grain-weight increased by 48.37%.

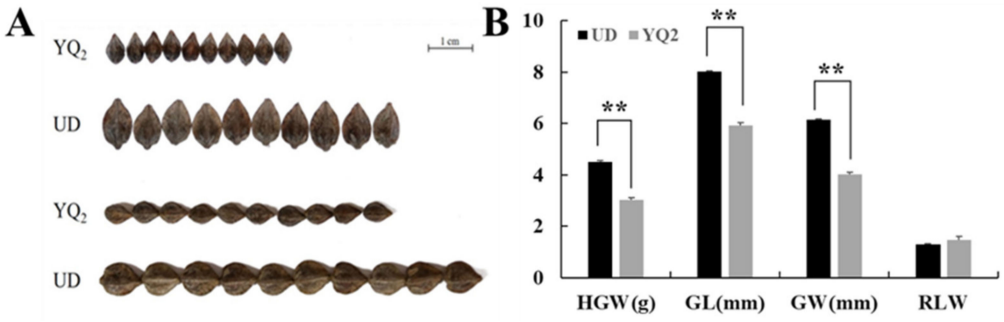

Figure 1. Comparison of mature grain size of parents. (**A**) Phenotypic observation. (**B**) Statistical analysis. GL for grain length, GW for grain width, HGW for hundred-grain weight, RLW for ratio of length-width. ** Significances with the probability levels of 0.01.

Statistical analysis of grain size-related parental traits and the F_1 population of 217 individuals were performed (Table 1). All traits were observed to segregate continuously. Compared with the parent, the maximum value of grain length, width, and hundred-grain weight in the F_1 population is bigger than UD, and its minimum value is smaller than YQ_2. Transgressive segregation was observed for all traits. The frequency histogram showed that these traits are all approximately normally distributed (Figure 2).

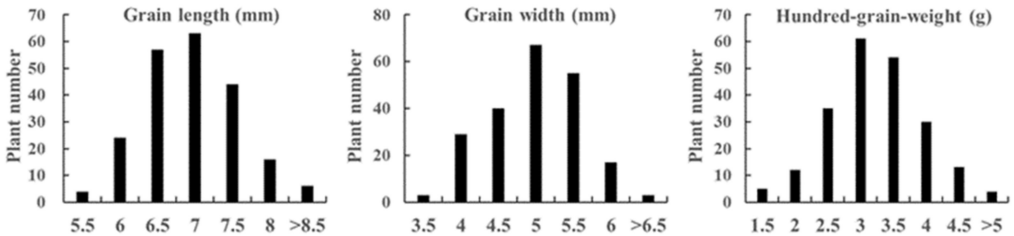

Figure 2. The frequency distribution of grain size traits in the F_1 population.

Table 1. Phenotypic data analysis of grain size traits for the parents and F_1 populations.

		Hundred-Grain-Weight (g)	Grain Length (mm)	Grain Width (mm)
Parent	UD	4.500 ± 0.06 **	8.02 ± 0.03 **	6.15 ± 0.01 **
	YQ2	3.033 ± 0.09	5.92 ± 0.11	4.02 ± 0.08
F_1	Mean	3.060	6.81	4.95
	Max	4.767	9.43	7.30
	Min	1.200	5.00	3.23
	Skewness	−0.119	0.23	0.20
	Kurtosis	0.052	0.94	0.97

** Significances with the probability levels of 0.01.

3.2. Development of Primer

At the early stage of the project team, a transcriptome sequence was carried out on 5 and 10 days grains of parent varieties UD and YQ2 to identify the differentially expressed genes related to grain size in common buckwheat [36]. In this study, 187,034 unigenes obtained by transcriptome sequencing were searched for SSR sites, and 24,609 EST-SSR sites were found from 21,376 unigene. The distribution frequency of SSR in the transcriptome was 13.16% (Table S2). A total of 18,968 pairs of EST-SSR primers were designed and developed by setting the length of primers to 18–23 bp and the size of amplification products to 100–300 bp (Table S3). Among these, 100 pairs of SSR primers were randomly synthesized for constructing a genetic linkage map.

The transcriptome data were analyzed by mutation detection software to search the InDel sites, and 31,225 InDel sites were found from 187,034 Unigene (Table S4). The InDel length 0–4 bp were most abundant (24,494), with a proportion of 78.44%, followed by 5–10 bp (4602, 14.74%), length 11–20 bp (1298, 4.16%), length ≥ 21 bp (831, 2.66%) (Table S2). To facilitate the analysis of PCR products by polypropylene gel electrophoresis, InDel sites with 5–10 bp were selected for primer design. A total of 312 pairs of InDel primers were designed and synthesized, with a product size of 100–300 bp.

3.3. Construction of Genetic Linkage Map

A total of 320 pairs of SSR primers and 336 pairs of InDel primers were screened between the two parents (UD and YQ2). A total of 80 pairs of polymorphism primers were obtained from EST-SSR primers with a polymorphism ratio of 25.0%, and 145 pairs of polymorphism primers were obtained from InDel primers with a polymorphism ratio of 43.2%. The marker genotypes of 217 individual strains in the F_1 population (UD and YQ_2) were detected by polymorphism primers, and 265 loci were obtained. According to JoinMap 4.0 requirements for the "CP drawing model" (Van Ooijen, 2006), the different segregation types are labeled as hk × hk, lm × ll, nn × np, ef × eg, ab × cd (Figure 3).

Among the 265 polymorphism loci, 166 loci conformed to the requirement of the pseudo-testcross mapping strategy (Table 2). Based on the linkage analysis of 166 loci, a genetic linkage map with 132 loci was constructed (Figure 4, Table 3). There were 8 linkage groups in total, including 39 SSR loci (including four double loci markers) and 93 InDel loci, with a total length of 1398.33 cM and an average spacing of 10.59 cM.

Table 2. Loci number of the different segregation types.

Segregation Types	EST-SSR Loci		InDel Loci		Total
	Number	Proportion (%)	Number	Proportion (%)	
hk × hk	33	29.20	80	70.80	113
nn × np	12	46.15	14	53.90	26
lm × ll	8	44.44	10	55.56	18
ef × eg	1	11.11	8	88.89	9
ab × cd	0	100.00	0	100.00	0

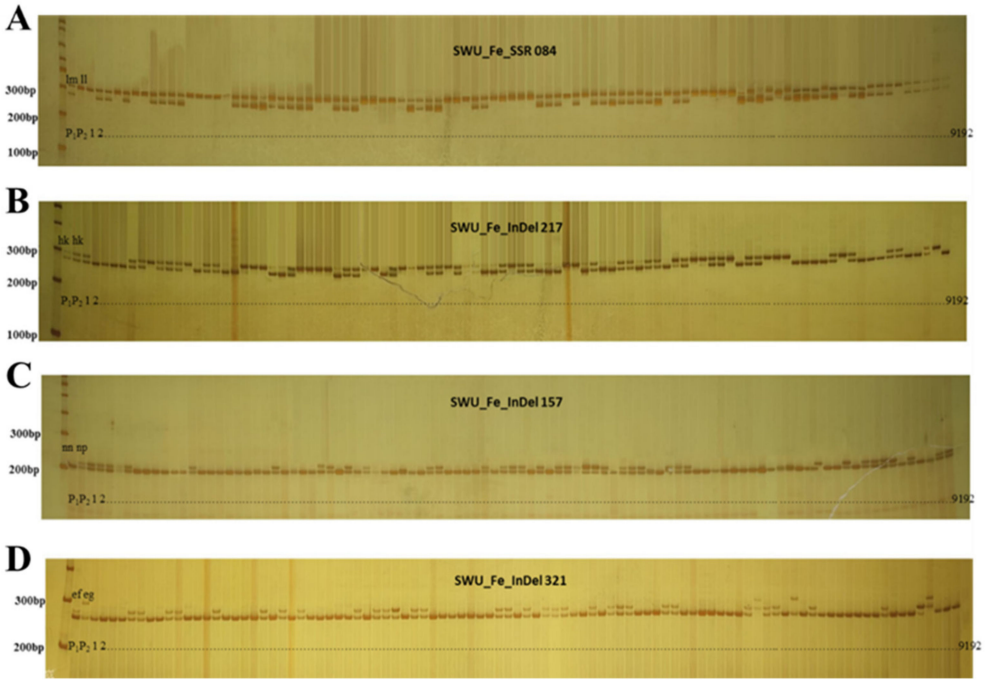

Figure 3. Electrophoregram of PCR products for F_1 individuals. P_1: Youqiao2; P_2: Ukraine daliqiao; 1–92: Individuals from the F_1 population. (**A**) segregation type lm × ll; (**B**) hk × hk; (**C**) nn × np; (**D**) ef × eg.

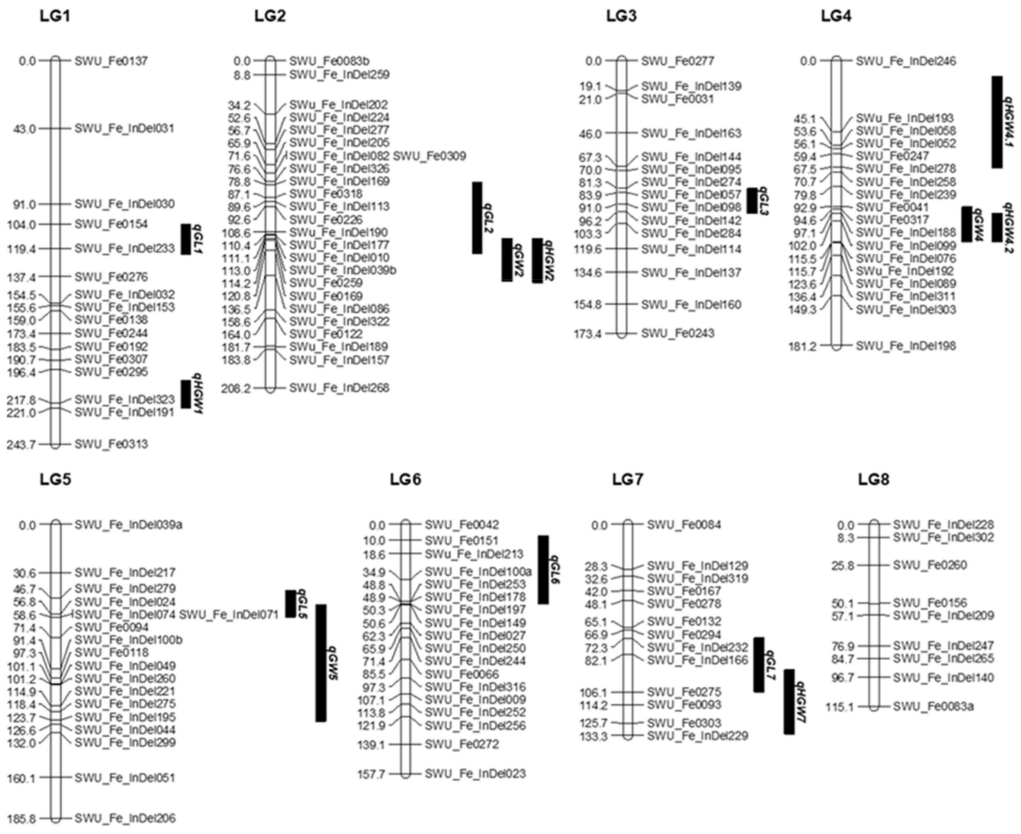

Figure 4. The Genetic linkage map and QTL controlling grain size traits from (UD × YQ2) F_1 population. The QTL controlling grain size traits and the bars representing 1-LOD likelihood intervals are beside the linkage group. QTL is shown as GL for grain length, GW for grain width, and HGW for hundred-grain weight.

Table 3. Distribution of loci on the linkage maps from the F_1 population.

Group	Length/cM	Total of Loci	No of EST-SSR	No of InDel	Average Intervals (cM)
LG1	243.75	16	9	7	15.23
LG 2	208.24	25	7	18	8.33
LG 3	173.37	15	3	12	11.56
LG 4	181.19	18	3	15	10.07
LG 5	185.76	18	2	16	10.32
LG 6	157.67	18	4	14	8.76
LG 7	133.27	13	8	5	10.25
LG 8	115.09	9	3	6	12.79
Total	1398.33	132	39	93	10.59

3.4. Preliminary Mapping of QTL Related to Grain Size Traits

Based on the phenotypic data of F_1 generation, combined with its genetic linkage map of common buckwheat, QTL for grain size-related traits in common buckwheat were mapped. A total of 14 QTLs were detected for grain size-related traits, including 6-grain length QTL, 3-grain width QTL, and 5 hundred-grain-weight QTL (Figure 4). The percentage of phenotypic variance explained by each QTL for each trait ranged from 4.6 (qHGW4.2) to 11.9 (qHGW7). According to the statistical analysis, the QTL of grain width and hundred-grain-weight were all detected near SWU_Fe_InDel086 in group 2 and SWU_Fe_InDel076 in group 4 (Table 4).

Table 4. QTL controlling grain size traits identified from the F_1 population.

Traits	QTL	Group	Nearest Marker	LOD	Var%	A
Grain length	qGL1	LG1	SWU_Fe_InDel233	3.13	6.8	0.622
	qGL2	LG2	SWU_Fe_InDel113	2.91	6.3	0.927
	qGL3	LG3	SWU_Fe_InDel098	2.12	4.7	0.735
	qGL5	LG5	SWU_Fe_InDel279	2.85	6.2	0.637
	qGL6	LG6	SWU_Fe_InDel213	3.39	8	0.849
	qGL7	LG7	SWU_Fe_InDel166	2.74	6	0.628
Grain width	**qGW2**	**LG2**	**SWU_Fe_ InDel086**	**3.04**	**6.6**	**0.74**
	qGW4	**LG4**	**SWU_Fe_InDel076**	**2.61**	**5.7**	**0.618**
	qGW5	LG5	SWU_Fe_InDel195	3.23	7	0.665
Hundred-grain-weight	qHGW1	LG1	SWU_Fe_InDel323	3.43	7.4	0.705
	qHGW2	**LG2**	**SWU_Fe_InDel086**	**5.04**	**10.7**	**0.795**
	qHGW4.1	LG4	SWU_Fe_InDel278	2.9	6.3	0.782
	qHGW4.2	**LG4**	**SWU_Fe_InDel076**	**2.09**	**4.6**	**0.618**
	qHGW7	LG7	SWU_Fe0303	5.66	11.9	0.847

Bold figures indicate the QTL was detected in more than two traits simultaneously. Var%, Phenotypic variation explained by a single QTL. A, additive effect.

3.5. Candidate Gene Screening

Based on the transcriptome sequencing data of the parents [36] (accession: PRJNA523295), the expression of unigenes corresponding to the markers located in the QTL region was analyzed (Table S5, Figure 5). There were no significant DEGs between parents UD and YQ2. However, the expression of *Cluster-3342.67767* (SWU_Fe_InDel086), *Cluster-28557.1* (SWU_Fe_InDel098), and *Cluster-22733.0* (SWU_Fe_InDel278) had a high expression of grain, and there was more than a one-fold increase in the 5 DPA of UD, compared with YQ2. In addition, *Cluster-3342.104850* (SWU_Fe_InDel323), *Cluster-3342.75374* (SWU_Fe_InDel191), *Cluster-3342.49496* (SWU_Fe_InDel169), and *Cluster-3342.80450* (SWU_Fe_InDel113) had a higher expression in 5 DPA and 10 DPA grains of common buckwheat, even though there was no expression difference, but with an InDel difference between parents.

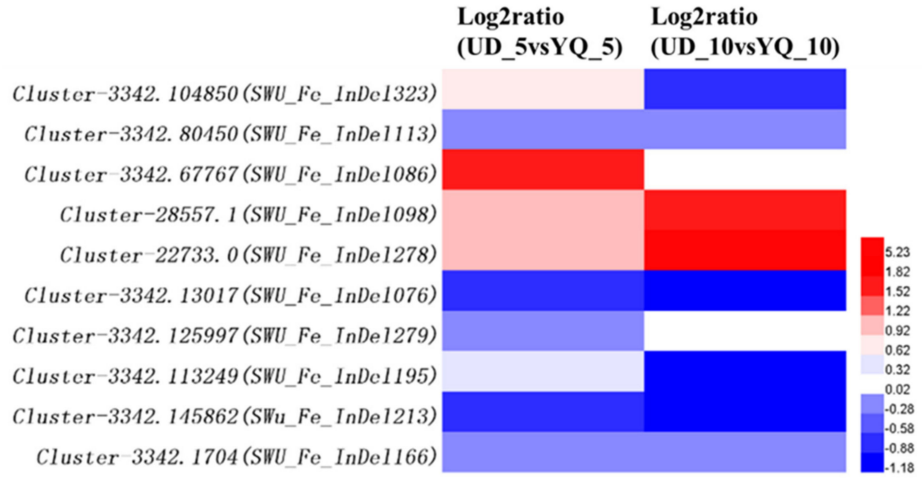

Figure 5. Expression analysis of candidate genes corresponding to nearest markers located in QTL region.

4. Discussion

4.1. Development of Molecular Markers in Common Buckwheat

Molecular markers play a very important role in the genetic diversity analysis for natural populations, genetic map construction, QTL mapping of major traits, and MAS breeding [43]. SSR markers are also the main types of molecular markers due to their wide distribution and simple application. So far, hundreds of plant species have constructed genetic maps containing SSR markers. Although the draft genome sequence of common buckwheat has been published [44], there are still few SSR markers available in common buckwheat [19–21,45]. In this study, 18,968 pairs of EST-SSR primers were developed by transcriptome sequencing and successfully used for genetic map construction. The large set of EST-SSR primers had a great value owing to their higher level of transferability to relate to species. They could often be used for constructing high-density genetic maps for positional gene cloning, association analysis, and detailed comparative mapping in Fagopyrum species.

Insertions and deletions (InDels) are the most abundant structural variation in genomes. They have been recognized as an important source of molecular markers due to their high density, ease of genotyping, and cost-effectiveness [46]. In recent years, the rapid development of high-throughput sequencing technology also provides convenience for the development and utilization of InDel markers. There have been more and more reports on the application of InDel markers in genetic map construction in watermelon [47], rice [48], soybean [49], Sichuan pepper [50]. In this study, 31,225 InDel loci were found in 187,034 Unigene, and 326 pairs of InDel primers were synthesized. A total of 133 pairs of polymorphic primers were obtained with a 39.58% polymorphism ratio. The low polymorphism may be due to the heterozygosity of the parents, and the omission of parental screening, such as hk × hk type. On the other hand, it may be that the transcriptome sequencing in this study was without reference, which was different from the sequence of buckwheat itself.

4.2. Construction of Genetic Map in Common Buckwheat

Since common buckwheat is self-incompatible, it is difficult to use conventional population (F_2, BC_1, RILs) and conventional methods for genetic map construction. The pseudo-testcross strategy provided an effective method for constructing a genetic linkage map for common buckwheat. Some studies have confirmed the usefulness of linkage maps, even for allogamous populations [15,19,24]. In the present study, we constructed a genetic linkage map of common buckwheat using the pseudo-testcross strategy, including 39 SSR loci and 93 InDel loci, with a total length of 1398.33 cM and an average spacing of 10.06 cM. Here, the genetic map constructed in the present study is the most detailed common buckwheat intraspecific map based on SSR and InDel markers to date, which

could be used to construct a detailed consensus map or as a reference genetic map for common buckwheat genome assembly.

Although a draft genome sequence of common buckwheat (FES_r 1.0) with a total length of 1,177,687,305 bp has been published, this draft is fragmented and contains 387,597 scaffolds [44]. At present, there has not been a set of specific, efficient, and stable marker systems, and the lack of an accurate high-density genetic map seriously restricts the research of buckwheat genomics. In this study, all the molecular markers in the constructed genetic linkage map were anchored in scaffolds of the Common buckwheat genome sequence [44] and chromosome of the Tartary buckwheat genome sequence [51] (Table S6), and 132 marker sites were successfully anchored to 120 Scaffolds of the Common buckwheat genome. There were also seven scaffolds anchored by two markers and two scaffolds anchored by three markers. This is the first genetic linkage map of marker loci anchored by genome sequence in buckwheat, which is of great significance for the genomics of buckwheat and meta-analysis of QTL.

4.3. QTL Mapping and Candidate Genes Screening for Grain Size Related Traits

Buckwheat is grown worldwide and can withstand various environmental conditions, particularly a wide range of temperatures and photoperiods. As a newly-developing crop with rich nutritional value, the research progress of common buckwheat on molecular genetics and breeding is not as fast as that of other major crops. The development of molecular markers, the construction of genetic linkage maps, and the QTL mapping of main traits are lagging. The QTL mapping and candidate genes screening of yield traits is rarely reported. In the present study, 14 QTL of common buckwheat grain size-related traits were detected, including 6-grain length QTL, 3-grain width QTL, and 5 hundred-grain-weight QTL, which will be valuable for map-based cloning of yield traits and molecular breeding in high-yield common buckwheat.

More importantly, the expression of unigenes corresponding to the markers located in the QTL region was analyzed based on the transcriptome sequencing data of the parents [36]. Some putative candidate genes associated with grain size were identified within the QTL interval. *Cluster-3342.67767* (SWU_Fe_InDel086) encoding histone acetyltransferase could be homologous to GW6a, which interacts with ubiquitin-interacting motif-type ubiquitin receptor HDR3 to control the grain size in rice [52]. *OsCOMT* (caffeic acid O-methyltransferase) increases rice grain yield through the dual regulation of leaf senescence and vascular development [53]. In the present study, *Cluster-3342.49496* (SWU_Fe_InDel169) encodes methyltransferase and had higher expression in 5 DPA and 10 DPA grain, which could be involved in the grain size development in common buckwheat. In addition, the other unigenes could be involved in the grain size of common buckwheat owing to the InDel difference between parents, especially *Cluster-3342.104850* (SWU_Fe_InDel323), *Cluster-3342.80450* (SWU_Fe_InDel113), *Cluster-28557.1* (SWU_Fe_InDel098), *Cluster-22733.0* (SWU_Fe_InDel278) and *Cluster-3342.113249* (SWU_Fe_InDel195), with high expression in grain and nearest markers. All the QTL and candidate genes will provide the theoretical reference for the further map-based cloning and molecular mechanism of grain size in common buckwheat.

5. Conclusions

Based on an F_1 population segregated from a cross between UD and YQ that showed a significant difference in grain size, we constructed a genetic linkage map using the pseudo-testcross strategy, including 39 SSR loci and 93 InDel loci, which were developed from transcriptome sequencing between parents. A total of 14 QTL were detected, including 6-grain length QTL, 3-grain width QTL, and 5 hundred-grain-weight QTL. Marker-assisted breeding can be employed to transfer this QTL to improve grain size in common buckwheat. Seven putative candidate genes associated with grain size were identified within the QTL interval. Further investigation should be carried out to validate the exact gene for map-

based cloning, the molecular mechanism of grain size, and utilization to improve the yield of common buckwheat.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092062/s1, Table S1: 100 SSR, and 312 InDel primer synthesized in this study. Table S2: Summary of SSR and InDel identified in the *F. esculentum* transcriptome. Table S3: Details of SSR and primer identified in the *F. esculentum* transcriptome. Table S4: Details of InDel identified in the *F. esculentum* transcriptome. Table S5: Expression and functional annotation of unigenes corresponding to markers located in the QTL region. Table S6: The physical positions of markers located on genetic maps on the genomes of Common buckwheat and Tartary buckwheat.

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References

1. Zhang, H.; Li, Y.; Zhu, J.-K. Developing naturally stress-resistant crops for a sustainable agriculture. *Nat. Plants* **2018**, *4*, 989–996. [CrossRef] [PubMed]
2. Mir, N.A.; Riar, C.S.; Singh, S. Nutritional constituents of pseudo cereals and their potential use in food systems: A review. *Trends Food Sci. Technol.* **2018**, *75*, 170–180. [CrossRef]
3. Sytar, O.; Brestic, M.; Zivcak, M.; Tran, L.-S.P. The Contribution of Buckwheat Genetic Resources to Health and Dietary Diversity. *Curr. Genom.* **2016**, *17*, 193–206. [CrossRef]
4. Giménez-Bastida, J.A.; Zieliński, H. Buckwheat as a functional food and its effects on health. *J. Agric. Food Chem.* **2015**, *63*, 7896–7913. [CrossRef]
5. Fabjan, N.; Rode, J.; Kosir, I.J.; Wang, Z.; Zhang, Z.; Kreft, I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *J. Agric. Food Chem.* **2003**, *51*, 6452–6455. [CrossRef]
6. Bonafaccia, G.; Marocchini, M.; Kreft, I. Composition and technological properties of the flour and bran from common and Tartary buckwheat. *Food Chem.* **2003**, *80*, 9–15. [CrossRef]
7. Lewis, D.; Jones, D.A. The Genetics of Heterostyly. In *Evolution and Function of Heterostyly*; Barrett, S.C.H., Ed.; Springer: Berlin, Germany, 1992; pp. 129–150.
8. Campbell, C.G. *Buckwheat, Fagopyrum esculentum Moench*; Bioversity International: Rome, Italy, 1997; Volume 19.
9. Acquaah, G. *Principles of Plant Genetics and Breeding*; John Wiley & Sons: West Sussex, UK, 2009.
10. Yano, K.; Ohsawa, R.; Yonezawa, K. Cost Efficiency of Spatial Error Control in Single Plant Selection. *Breed. Sci.* **2002**, *52*, 177–184. [CrossRef]
11. Kump, B.; Javornik, B. Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. *Plant Sci.* **1996**, *114*, 149–158. [CrossRef]
12. Aii, J.; Nagano, M.; Penner, A.G.; Campbell, G.C.; Adachi, T. Identification of RAPD markers linked to the homostylar (Ho) gene in buckwheat. *Breed. Sci.* **1998**, *48*, 59–62. [CrossRef]
13. Suvorova, G.N.; Funatsuki, H.; Terami, F. Phylogenetic relationships among cultivars, species, and hybrids in the genus *Fagopyrum* Mill. assessed by RAPD analysis. *Russ. J. Genet.* **1999**, *35*, 1428–1432.
14. Sharma, T.; Jana, S. Species relationships in Fagopyrum revealed by PCR-based DNA fingerprinting. *Theor. Appl. Genet.* **2002**, *105*, 306–312. [CrossRef] [PubMed]

15. Yasui, Y.; Wang, Y.; Ohnishi, O.; Campbell, C.G. Amplified fragment length polymorphism linkage analysis of common buckwheat (*Fagopyrum esculentum*) and its wild self-pollinated relative *Fagopyrum homotropicum*. *Genome* **2004**, *47*, 345–351. [CrossRef]
16. Matsui, K.; Kiryu, Y.; Komatsuda, T.; Kurauchi, N.; Ohtani, T.; Tetsuka, T. Identification of AFLP makers linked to non-seed shattering locus (sht1) in buckwheat and conversion to STS markers for marker-assisted selection. *Genome* **2004**, *47*, 469–474. [CrossRef] [PubMed]
17. Konishi, T.; Yasui, Y.; Ohnishi, O. Original birthplace of cultivated common buckwheat inferred from genetic relationships among cultivated populations and natural populations of wild common buckwheat revealed by AFLP analysis. *Genes Genet. Syst.* **2005**, *80*, 113–119. [CrossRef] [PubMed]
18. Iwata, H.; Imon, K.; Tsumura, Y.; Ohsawa, R. Genetic diversity among Japanese indigenous common buckwheat (*Fagopyrum esculentum*) cultivars as determined from amplified fragment length polymorphism and simple sequence repeat markers and quantitative agronomic traits. *Genome* **2005**, *48*, 367–377. [CrossRef]
19. Konishi, T.; Ohnishi, O. A linkage map for common buckwheat based on microsatellite and AFLP markers. *Fagopyrum* **2006**, *23*, 1–6.
20. Ma, K.H.; Kim, N.S.; Lee, G.A.; Lee, S.Y.; Lee, J.K.; Yi, J.Y.; Park, Y.J.; Kim, T.S.; Gwag, J.G.; Kwon, S.J. Development of SSR markers for studies of diversity in the genus Fagopyrum. *Theor. Appl. Genet.* **2009**, *119*, 1247–1254. [CrossRef]
21. Pan, S.-J.; Chen, Q.-F. Genetic mapping of common buckwheat using DNA, protein and morphological markers. *Hereditas* **2010**, *147*, 27–33. [CrossRef]
22. Shi, T.X.; Li, R.Y.; Chen, Q.J.; Li, Y.; Pan, F.; Chen, Q.F. De novo sequencing of seed transcriptome and development of genic-SSR markers in common buckwheat (*Fagopyrum esculentum*). *Mol Breed.* **2017**, *37*, 147. [CrossRef]
23. Hara, T.; Iwata, H.; Okuno, K.; Matsui, K.; Ohsawa, R. QTL analysis of photoperiod sensitivity in common buckwheat by using markers for expressed sequence tags and photoperiod-sensitivity candidate genes. *Breed. Sci.* **2011**, *61*, 394–404. [CrossRef]
24. Yabe, S.; Hara, T.; Ueno, M.; Enoki, H.; Kimura, T.; Nishimura, S.; Yasui, Y.; Ohsawa, R.; Iwata, H. Rapid genotyping with DNA micro-arrays for high-density linkage mapping and QTL mapping in common buckwheat (*Fagopyrum esculentum* Moench). *Breed. Sci.* **2014**, *64*, 291–299. [CrossRef] [PubMed]
25. Matsui, K.; Mizuno, N.; Ueno, M.; Takeshima, R.; Yasui, Y. Development of co-dominant markers linked to a hemizygous region that is related to the self-compatibility locus (S) in buckwheat (*Fagopyrum esculentum*). *Breed. Sci.* **2020**, *70*, 112–117. [CrossRef] [PubMed]
26. Grattapaglia, D.; Sederoff, R. Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudotestcross: Mapping strategy and RAPD markers. *Genetics* **1994**, *137*, 1121–1137. [CrossRef] [PubMed]
27. Serba, D.D.; Sykes, R.W.; Gjersing, E.L.; Decker, S.R.; Daverdlin, G.; Devos, K.M.; Brummer, E.C.; Saha, M.C. Cell Wall Composition and Underlying QTL in an F 1 Pseudo-Testcross Population of Switchgrass. *Bioenerg. Res.* **2016**, *9*, 836–850. [CrossRef]
28. Xu, J.M.; Fan, W.; Jin, J.F.; Lou, H.Q.; Chen, W.W.; Yang, J.L.; Zheng, S.J. Transcriptome analysis of Al-induced genes in buckwheat (*Fagopyrum esculentum* Moench) root apex: New insight into Al toxicity and resistance mechanisms in an Al accumulating species. *Front. Plant Sci.* **2017**, *8*, 1141. [CrossRef]
29. Koech, R.K.; Malebe, P.M.; Nyarukowa, C.; Mose, R.; Kamunya, S.M.; Apostolides, Z. Identification of novel QTL for black tea quality traits and drought tolerance in tea plants (*Camellia sinensis*). *Tree Genet. Genomes* **2018**, *14*, 9. [CrossRef]
30. Yang, X.P.; Sood, S.; Glynn, N.; Islam, M.S.; Comstock, J.; Wang, J.P. Constructing high-density genetic maps for polyploid sugarcane (*Saccharum* spp.) and identifying quantitative trait loci controlling brown rust resistance. *Mol. Breed.* **2017**, *37*, 116. [CrossRef]
31. McClure, K.A.; Gardner, K.M.; Toivonen, P.M.A.; Hampson, C.R.; Song, J.; Forney, C.F.; DeLong, J.; Rajcan, I.; Myles, S. QTL analysis of soft scald in two apple populations. *Hortic. Res.* **2016**, *3*, 16043. [CrossRef]
32. Liu, T.; Guo, L.L.; Pan, Y.L.; Zhao, Q.; Wang, J.H.; Song, Z.Q. Construction of the first high-density genetic linkage map of *Salvia miltiorrhiza* using specific length amplified fragment (SLAF) sequencing. *Sci. Rep.* **2016**, *6*, 24070. [CrossRef]
33. Yabe, S.; Hara, T.; Ueno, M.; Enoki, H.; Kimura, T.; Nishimura, S.; Yasui, Y.; Ohsawa, R.; Iwata, H. Potential of genomic selection in mass selection breeding of an allogamous crop: An empirical study to increase yield of common buckwheat. *Front. Plant Sci.* **2018**, *9*, 276. [CrossRef]
34. Hara, T.; Takeshima, R.; Matsui, K. Genes with different modes of inheritance regulate seed germination in preharvest-sprouting-tolerant lines of buckwheat (*Fagopyrum esculentum*). *Jpn. Agric. Res. Q* **2020**, *54*, 137–143. [CrossRef]
35. Takeshima, R.; Ogiso, T.E.; Yasui, Y.; Matsui, K. Targeted amplicon sequencing + next-generation sequencing–based bulked segregant analysis identified genetic loci associated with preharvest sprouting tolerance in common buckwheat (*Fagopyrum esculentum*). *BMC Plant Biol.* **2021**, *21*, 18. [CrossRef]
36. Fang, X.M.; Zhang, Y.L.; Zhang, Y.K.; Huang, K.H.; Yang, W.J.; Li, X.Y.; Zhang, Z.Y.; Wu, K.H.; Xu, X.; Ruan, R.W.; et al. De novo transcriptome assembly and identification of genes related to seed size in common buckwheat (*Fagopyrum esculentum* M.). *Breed. Sci.* **2019**, *69*, 487–497. [CrossRef] [PubMed]
37. Liu, Y.; Fang, X.M.; Tang, T.; Wang, Y.D.; Wu, Y.H.; Luo, J.Y.; Wu, H.T.; Wang, Y.Q.; Zhang, J.; Ruan, R.W.; et al. Inflorescence Transcriptome Sequencing and Development of New EST-SSR Markers in Common Buckwheat (*Fagopyrum esculentum*). *Plants* **2022**, *11*, 742. [CrossRef] [PubMed]

38. Li, H.; Handsaker, B.; Wysoker, A.; Fennell, T.; Ruan, J.; Homer, N.; Marth, G.; Abecasis, G.; Durbin, R.; 1000 Genome Project Data Processing Subgroup (Subgroup GPDP). The sequence alignment/map format and SAMtools. *Bioinformatics* **2009**, *25*, 2078–2079. [CrossRef]
39. Zhang, Z.S.; Xiao, Y.H.; Luo, M.; Li, X.B.; Luo, X.Y.; Hou, L.; Li, D.M.; Pei, Y. Construction of a genetic linkage map and QTL analysis of fiber-related traits in upland cotton (*Gossypium hirsutum* L.). *Euphytica* **2005**, *144*, 91–99. [CrossRef]
40. Van Ooijen, J.W.; Voorrips, R.E. *JoinMap 4.0., Software for the Calculation of Genetic Linkage Maps*; Plant Research International: Wageningen, The Netherlands, 2006.
41. Van Ooijen, J.W. *MapQTL 6.0., Software for the Mapping of Quantitative Trait Loci in Experimental Populations*; Plant Research International: Wageningen, The Netherlands, 2009.
42. Voorrips, R.E. *MapChart 2.2: Software for the Graphical Presentation of Linkage Maps and QTLs*; Plant Research International: Wageningen, The Netherlands, 2006.
43. Varshney, R.K.; Graner, A.; Sorrells, M.E. Genic microsatellite markers in plants: Features and applications. *Trends Biotechnol.* **2005**, *23*, 48–55. [CrossRef]
44. Yasui, Y.; Hirakawa, H.; Ueno, M.; Matsui, K.; Katsube-Tanaka, T.; Yang, S.J.; Aii, J.; Sato, S.; Mori, M. Assembly of the draft genome of buckwheat and its applications in identifying agronomically useful genes. *DNA Res.* **2016**, *23*, 215–224. [CrossRef]
45. Hou, S.Y.; Sun, Z.X.; Bin, L.H.; Xu, D.M.; Wu, B.; Zhang, B.; Wang, X.C.; Han, Y.H.; Zhang, L.J.; Qiao, Z.J.; et al. Genetic diversity of buckwheat cultivars (*Fagopyrum tartaricum* Gaertn.) assessed with SSR markers developed from genome survey sequences. *Plant Mollecular Biol. Report.* **2016**, *34*, 233–241. [CrossRef]
46. Liu, J.; Qu, J.T.; Yang, C.; Tang, D.G.; Li, J.W.; Lan, H.; Rong, T.Z. Development of genome-wide insertion and deletion markers for maize, based on next-generation sequencing data. *BMC Genom.* **2015**, *16*, 601. [CrossRef]
47. Ren, Y.; Zhao, H.; Kou, Q.H.; Jiang, J.; Guo, S.G.; Zhang, H.Y.; Hou, W.J.; Zou, X.H.; Sun, H.H.; Gong, G.Y.; et al. A High Resolution Genetic Map Anchoring Scaffolds of the Sequenced Watermelon Genome. *PLoS ONE* **2012**, *7*, e29453. [CrossRef] [PubMed]
48. Wu, D.H.; Wu, H.P.; Wang, C.S.; Tseng, H.Y.; Hwu, K.K. Genome-wide InDel marker system for application in rice breeding and mapping studies. *Euphytica* **2013**, *192*, 131–143. [CrossRef]
49. Lu, S.J.; Li, Y.; Wang, J.L.; Srinives, R.; Nan, H.Y.; Cao, D.; Wang, Y.P.; Li, J.L.; Li, X.M.; Fang, C.; et al. QTL mapping for flowering time in different latitude in soybean. *Euphytica* **2015**, *206*, 725–736. [CrossRef]
50. Tan, S.; Cheng, J.W.; Zhang, L.; Cheng, Q.; Nong, D.G.; Li, W.P.; Tang, X.; Wu, Z.M.; Hu, K.L. Construction of an Interspecific Genetic Map Based on InDel and SSR for Mapping the QTLs Affecting the Initiation of Flower Primordia in Pepper (*Capsicum* spp.). *PLoS ONE* **2015**, *10*, e0119389. [CrossRef] [PubMed]
51. Zhang, L.J.; Li, X.X.; Ma, B.; Gao, Q.; Du, H.L.; Han, Y.H.; Li, Y.; Cao, Y.H.; Qi, M.; Zhu, Y.X.; et al. The Tartary Buckwheat Genome Provides Insights into Rutin Biosynthesis and Abiotic Stress Tolerance. *Mol. Plant* **2017**, *10*, 1224–1237. [CrossRef]
52. Gao, Q.; Zhang, N.; Wang, W.Q.; Shen, S.Y.; Bai, C.; Song, X.J. The ubiquitin-interacting motif-type ubiquitin receptor HDR3 interacts with and stabilizes the histone acetyltransferase GW6a to control the grain size in rice. *Plant Cell* **2021**, *33*, 3331–3347. [CrossRef]
53. Huangfu, L.X.; Chen, R.J.; Lu, Y.; Zhang, E.Y.; Miao, J.; Zuo, Z.H.; Zhao, Y.; Zhu, M.Y.; Zhang, Z.H.; Li, P.C.; et al. *OsCOMT*, encoding a caffeic acid O-methyltransferase in melatonin biosynthesis, increases rice grain yield through dual regulation of leaf senescence and vascular development. *Plant Biotechnol. J.* **2022**, *20*, 1122–1139. [CrossRef]