Genome-Wide Association Mapping Identifies New Candidate Genes for Cold Stress and Chilling Acclimation at Seedling Stage in Rice (Oryza sativa L.)

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Abstract: Rice (Oryza sativa L.) is a chilling-sensitive staple food crop, and thus, low temperature significantly affects rice growth and yield. Many studies have focused on the cold shock of rice although chilling acclimation is more likely to happen in the field. In this paper, a genome-wide association study (GWAS) was used to identify the genes that participated in cold stress and chilling accumulation. A total of 235 significantly associated single-nucleotide polymorphisms (SNPs) were identified. Among them, we detected 120 and 88 SNPs for the relative shoot fresh weight under cold stress and chilling acclimation, respectively. Furthermore, 11 and 12 quantitative trait loci (QTLs) were identified for cold stress and chilling acclimation, respectively, by integrating the co-localized SNPs. Interestingly, we identified 10 and 15 candidate genes in 11 and 12 QTLs involved in cold stress and chilling acclimation, respectively, and two new candidate genes (LOC_Os01g62410, LOC_Os12g24490) were obviously up-regulated under chilling acclimation. Furthermore, OsMYB3R-2 (LOC_Os01g62410) that encodes a R1R2R3 MYB gene was associated with cold tolerance, while a new C3HC4-type zinc finger protein-encoding gene LOC_Os12g24490 was found to function as a putative E3 ubiquitin-protein ligase in rice. Moreover, haplotype, distribution, and Wright’s fixation index (FST) of both genes showed that haplotype 3 of LOC_Os12g24490 is more stable in chilling acclimation, and the SNP (A > T) showed a difference in latitudinal distribution. FST analysis of SNPs in OsMYB3R-2 (LOC_Os01g62410) and LOC_Os12g24490 indicated that several SNPs were under selection in rice indica and japonica subspecies. This study provided new candidate genes in genetic improvement of chilling acclimation response in rice.

Keywords: rice; fresh weight; chilling acclimation; cold stress; GWAS; RNA-seq

1. Introduction

Rice (Oryza sativa) is one of the three major cereal crops used as a staple food by more than half of the world population [1]. As rice originated in tropical or subtropical areas, it is generally sensitive to cold stress [2,3]. Low-temperature stress is the main limiting factor of rice cultivation, as it affects crop yield and quality. Therefore, it is important to develop more robust and cold-stress-resilient rice germplasms. The identification of genes involved in cold tolerance is a crucial aspect of rice breeding.

Plants have evolved sophisticated regulatory networks to cope with temperature environments. Asian cultivated rice is represented by two subspecies, indica and japonica, and each of them includes many accessions exhibiting different tolerance levels to cold stress [4]; the underlying genetic variation has become a powerful tool for the discovery of many genetic loci and cold-related genes in rice using these genetic resources [5]. It is interesting to find out the physiological and molecular mechanisms underlying their differences in
plant responses to cold stresses, which can provide new power for engineering cold-tolerant and high-yielding rice varieties.

In addition, there are two kinds of low temperature in rice: (1) rapid decline of the temperature and (2) chilling stress, during which the temperature gradually declines to an unfavorable range for rice growth. Most studies of cold-stress responses in rice are close to the rapid decline of the temperature even though chilling acclimation is more likely to happen in rice growth and development. Therefore, it is necessary to study the mechanisms underlying chilling acclimation and find the candidate genes for the genetic improvement of rice cold tolerance.

In recent years, genome-wide association study (GWAS) has become an important method for identifying QTL and genomic regions in many species [6–8]. For example, Liu performed a GWAS of the tiller response to nitrogen that is most closely correlated with nitrogen-use efficiency in rice and identified a candidate gene OsTCP19 [9]. Overall, 51 QTLs were detected by genome-wide association study by using 174 Chinese rice accessions for cold tolerance [10]; 132 QTLs were detected in 527 rice cultivars for both rice natural chilling and cold shock stresses [11]; 56 novel loci were detected for cold stress at seedling stage [12], and a similar analysis was carried out by using a rice population of 2262 [13]. In recent studies, RNA-seq has been widely used in uncovering the causal genes in GWAS in plants [14,15].

To gain insight into the mechanisms that have enabled rice to endure cold environments, we performed a GWAS to elucidate the cold tolerance in rice. We collected 338 accessions of rice core germplasms and evaluated the performance of the rice seedling under two kinds of cold stress, i.e., cold shock and cold acclimation, by detecting the relative changes of shoot fresh weight, which is an ideal symbol for cold stress adopted by previous studies [16–19]. Fixed and random model circulating probability unification (FarmCPU) was also used in this study, which is one of the multi-locus models and is more robust in controlling both false positives and negatives [20,21]. In addition, RNA-seq data, haplotype, and distribution analysis were finally carried out for dissecting causal genes in GAWS. Thus, our study provides a further understanding of the genetic regulation for cold-stress tolerance in rice.

2. Results

2.1. Different Responses of Indica and Japonica Rice Seedlings and Trait Correlations under Different Temperature Conditions

In this study, shoot fresh weight was adopted to evaluate the cold response of the seedlings of 338 natural rice accessions under different temperature conditions (Supplementary Tables S1 and S2). Firstly, statistics of three traits were presented, including the mean, standard deviation (SD), minimum (min), maximum (max), coefficient of variation (CV), skewness, and kurtosis analyses, which were all carried out (Supplementary Table S3). The variation of the shoot fresh weight under normal temperature (SWNT) is relatively small. However, upon low-temperature treatments, a larger variation was observed, as indicated by the CV of the trait (Supplementary Table S3). Then, 338 rice accessions were allocated to three subpopulations (indica, japonica, and intermediate) according to previous study [22]. It was shown that the shoot fresh weight of japonica accessions was significantly higher than that of the indica accessions under normal temperature (Figure 1A). After low-temperature treatments, the relative shoot fresh weight of japonica was significantly lower than indica and intermediate accessions, suggesting that japonica accessions are more tolerant to cold stress than indica and intermediate accessions (Figure 1B,C). Correlation analysis indicated that relative shoot fresh weight after cold stress (RSWCS) and relative shoot fresh weight after chilling acclimation (RSWCA) are significantly and positively associated. Shoot fresh weight under normal temperature (SWNT) is not associated with that after cold stress and after chilling acclimation exposure (Supplementary Figure S1).
Principal component analysis (PCA) and kinship analysis, was performed and showed that with that after cold stress and after chilling acclimation exposure (Supplementary Figure S1). With that after cold stress and after chilling acclimation exposure (Supplementary Figure S1).

2.2. Population Genetic Analyses and Polymorphic SNPs

In this study, we collected 338 accessions consisting of 167 indica accessions, 48 intermediate accessions, and 123 japonica accessions (Figure 2A). Population structure analysis, including principal component analysis (PCA) and kinship analysis, was performed and showed that two components or clusters separate this panel (Figure 2B,C). Furthermore, the LD of this panel was estimated as 57 kb when \( r^2 \) dropped to half of the maximum value (Figure 2D) [23]. The genotypes of the 338 accessions were downloaded from the website https://snp-seek.irri.org, accessed on 22 June 2022 [24]. Then, about 3.4 Mb SNPs in the whole rice genome were filtered. In detail, chromosome 4 has the smallest marker density, with one SNP per 103 bp, while the largest marker density was observed on chromosome 10 (1 SNP/99 bp). The number of SNPs and marker density show little difference in 12 rice chromosomes. The average density of SNPs was one SNP per 111 bp (Supplementary Table S4).

![Figure 1](image1.png)

**Figure 1.** Comparison of shoot fresh weight of rice subpopulations under different temperature conditions. (A–C) The box plot of the response of three rice subpopulations seedlings to the normal temperature (A), cold stress (B), and chilling acclimation (C). SWNT (g), shoot fresh weight under normal temperature; RSWCS, relative fresh weight after cold stress; RSWCA, relative fresh weight after chilling acclimation; ind, indica subpopulation; inter, intermediate subpopulation; jap, japonica subpopulation. * and ** represent significant differences at the 0.05 and 0.01 levels, respectively.

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![Figure 2](image2.png)

**Figure 2.** Population genetic analyses and polymorphic SNPs. (A) Distribution of 338 rice accessions. (B) Principal component analysis of the association panel analyzed by PLINK [25]. (C) Kinship analysis of the association panel. (D) LD decay calculated by PopLDdecay.
2.3. GWAS of Shoot Fresh Weight under Three Different Conditions

Shoot fresh weight is an ideal symbol for detecting the relative changes of cold stress [16–19]. To obtain more reliable SNPs, FarmCPU was performed with the first two principal components and kinship by rMVP package [26]. Firstly, the $-\log(p)$ was set at 5 to identify significant association signals. Then, 235 SNPs were found for shoot fresh weight under normal temperature, cold stress, and chilling acclimation. In detail, 27 SNPs were identified for shoot fresh weight under normal temperature (Figure 3A and Supplementary Table S5), 120 SNPs were identified for relative shoot fresh weight under cold stress (Figure 3B and Supplementary Table S6), and 88 SNPs were identified for the relative shoot fresh weight under chilling acclimation (Figure 3C and Supplementary Table S7).

Figure 3. GWAS of the shoot fresh weight under three different conditions. (A–C) Manhattan plot of shoot fresh weight under normal temperature (SWNT (g)) (A), relative shoot fresh weight under cold stress (RSWCS)(B), and relative shoot weight under chilling acclimation (RSWCA)(C). The arrow is linked to significant SNPs that have been reported.

In addition, several significant SNPs located at the previously reported genes were annotated (Figure 3A–C). For example, OsHOX12 (LOC_Os03g10210) and OsLGG (LOC_Os11g41890) were detected for relative shoot fresh weight under cold stress, and both of them have been identified to be associated with panicle development [27,28]. As for the relative shoot fresh weight under chilling acclimation, OsMYB3R-2 (LOC_Os01g62410) was identified to be associated with cold tolerance [29], OsbZIP23 (LOC_Os02g52780) was involved in drought and salt tolerance [30], OsJMJ704 (LOC_Os05g23670) was a disease-resistance regulator [31], OsRBG1 (LOC_Os11g30430) enhanced tolerance to heat and osmotic and salt stress [32], and Ospita2 (LOC_Os12g18729) was also associated with disease resistance [33].
2.4. Comprehensive Analysis of Significant SNPs under Cold Stress and Chilling Acclimation Conditions

To obtain reliable results, regions with two or more significant SNPs were selected as QTL [34,35]. As a result, 11 QTLs and 12 QTLs were found for cold stress and chilling acclimation, respectively (Supplementary Table S8). For more details, 254 and 220 genes were found in 11 QTLs and 12 QTLs, respectively. Then, these genes were integrated with the RNA-seq data, and 10 and 15 genes of them were expressed in our previous RNA-seq data (Supplementary Table S9).

To filter the specific genes associated with chilling acclimation, a comprehensive analysis was carried out. Firstly, we compared SNPs between cold stress and chilling acclimation, and four overlaps were detected and shown in black triangles, suggesting that cold stress and chilling acclimation share several pathways, but most of them are different. In addition, two overlaps were found between normal temperature and chilling acclimation, indicating that these SNPs are not specific to chilling acclimation (Figure 4A). We can also find that 12 QTLs for chilling acclimation labeled with the black star are different from QTLs for cold stress (Figure 4B). Among fifteen genes in twelve QTLs, three genes have relatively higher expressions under chilling acclimation, and two up-regulated genes were selected for further study (Figure 4C). In addition, qRT-PCR was performed for the two candidate genes LOC_Os01g62410 and LOC_Os12g24490. As a result, both of them showed higher expression levels at cold stress (6°C) for 12 h and 24 h, and LOC_Os12g24490 was significantly up-regulated at 6°C for 12 h (Figure 4D).

2.5. LD Block, Haplotype, and Distribution Analyses of the Candidate Genes

LD block, haplotype, and distribution analyses were carried out for three selected high-confidence genes. The frequency of haplotypes in each subpopulation was checked, and only the haplotypes with more than twenty accessions were subjected to further analysis.

LD block analysis showed that SNPs within the gene OsMYB3R-2 (LOC_Os01g62410) have a higher LD (Figure 5A). The accessions harboring Hap_2 (mostly japonica type) has better performance under both cold stress and chilling acclimation than the others (Figure 5B). There were specific 14 SNPs in Hap_2, showing great differences between indica and japonica accessions (Figure 5C).

A haplotype network analysis indicated that Hap_1 consists of 112 indica and 21 intermediate accessions, Hap_3 consists of all indica accessions, while Hap_2 consists of 105 japonica accessions, three indica accessions, and five intermediate accessions. Moreover, Hap_4 consists of 13 intermediate accessions (Figure 5D). FST analysis showed that several SNPs in Hap_2 have a higher FST value between indica and japonica subpopulations, which indicated that these SNPs are under selection (Figure 5E).

As noted above, the gene LOC_Os12g24490 encodes a C3HC4-type domain containing protein. LD analysis showed that there is a higher linkage disequilibrium within the LOC_Os12g24490 (Figure 6A). Haplotype analysis showed that Hap_3 had the smallest relative shoot fresh weight under cold stress and chilling acclimation (Figure 6B). As expected, there was a specific SNP that occurred in Hap_3 at chr12:13981997 (Figure 6C). Distribution analysis of the gene LOC_Os12g24490 showed that chr12:13981997 has a higher FST (ind/jap), suggesting that chr12:13981997 is under selection (Figure 6F).
Figure 4. Integrated analysis of SNPs of the shoot fresh weight and expression pattern of genes in 12 QTLs. (A) Comparison of the QTL results under three different temperature conditions. (B) Distribution of SNPs on different chromosomes. Stars represent QTLs with two or more SNPs and that were specifically detected under chilling acclimation. (C) Expression pattern of 15 genes of 12 QTLs. (D) Relative expression of LOC_Os01g62410 and LOC_Os12g24490 by RT-qPCR.
**Figure 5.** LD block, haplotype, and distribution analyses of the SNPs. (A) LD block analysis of LOC_Os01g62410. (B) Haplotype analysis of LOC_Os01g62410 under cold stress and chilling acclimation. (C) SNPs information of each haplotype. (D) Haplotype network of LOC_Os01g62410 and color represents different rice subpopulations. White represents indica accessions, gray represents japonica accessions, and black represents intermediate accessions. (E) FST analysis of LOC_Os01g62410 between indica and japonica subpopulation. FST was calculated between indica and japonica accessions. A, color represents different rice subpopulations. Red represents indica accessions, blue represents japonica accessions, and the green represents intermediate accessions. ** represent significant differences at 0.01 levels.
Figure 6. LD block, haplotype, distribution, and FST analyses of the gene LOC_Os12g24490. (A) LD block analysis of the gene LOC_Os12g24490; (B) Haplotype analysis of LOC_Os12g24490 under cold stress and chilling acclimation, (C) SNPs information of each haplotype. (D) Distribution of the SNP in the gene LOC_Os12g24490. A, color represents different rice accessions. Red represents indica accessions, blue represents japonica accessions, and the green represents intermediate accessions. (E): Haplotype network of the gene LOC_Os12g24490, and color represents different rice subpopulations. White represents indica accessions, gray represents japonica accessions, and black represents intermediate accessions. (F): FST analysis of the gene LOC_Os12g24490 between indica and japonica subpopulations. FST was calculated between indica and japonica accessions. * and ** represent significant differences at the 0.05 and 0.01 levels, respectively.
3. Discussion

Cold stress and chilling acclimation are limiting factors for rice growth, development, and yield, and previous studies have focused on finding the cold-tolerance genes [36–38]. However, few studies have been performed for chilling acclimation, which more likely happens in natural environment.

In this study, we performed a comparative GWAS for cold stress and chilling acclimation. We found that most of the QTLs for cold stress and chilling acclimation are different, while there are also shared QTLs. Notably, we identified two genes, OsMYB3R-2 (LOC_Os01g62410) and LOC_Os12g24490, for chilling acclimation. Interestingly, OsMYB3R-2 could up-regulate the cell division genes such as OsCycB1;1 (LOC_Os01g59120), OsCycB2;1 (LOC_Os04g47580), OsCycB2;2 (LOC_Os06g51110), and OsCDC20.1 (LOC_Os02g47180) and enhance cold tolerance [29]. Therefore, OsMYB3R-2 (LOC_Os01g62410) may participate in both cold stress and chilling acclimation. Haplotype (network) analysis indicated that both OsMYB3R-2 (LOC_Os01g62410) and LOC_Os12g24490 diverged in indica and japonica. For example, Hap_2 of OsMYB3R-2 (LOC_Os01g62410) and Hap_3 of LOC_Os12g24490 mainly consisted of japonica accessions. According to FST analysis, we discovered that specific SNPs in Hap_2 of LOC_Os01g62410 had a higher FST value, indicating that these SNPs and haplotypes are under selection.

In our study, there are some QTLs overlapped with those identified in some previous studies, and several genes in QTLs have been verified to participate in cold stress or other abiotic stress. For example, qrswca5 overlapped with the QTL (chr4: 29737978-33358474) that contains the cold-tolerance gene OsAOX1a [39]. LOC_Os06g33710 in qrwcs7 encodes a distinctive class of spermidine synthase involved in chilling response in rice [40]. The LOC_Os06g37450 in qrswca6, a GATA transcription factor, confers cold tolerance by repressing OsWRKY45-1 at the seedling stage in rice [41]. Interestingly, several QTLs overlapped with drought-tolerance QTLs, such as qrswca8 and qrswca9 that overlapped with yld11.1 for yield per plant [42]; qrswca9 that overlapped with rv12-2 for root volume [43]; qrswca10 that overlapped with qtl12.1 for flowering delay; and qrswca1 that overlapped with qtl12.1 for drought-response index [44].

In recent years, GWAS has been widely used for identifying QTL and genomic regions of complex quantitative traits. As a routine, there were three important components: genotype, phenotype, and the model in GWAS. As for genotyping, the LD of rice is usually considered as 100 kb, which indicates that at least 4300 SNPs were required to cover the whole genome of rice (430 Mb) for GWAS. However, the increased number of SNPs will achieve higher accuracy of the QTLs mapping in GWAS. In this study, 3.4 M SNPs (average density is 111 bp/SNP) were used, which is efficient for GWAS mapping. Secondly, traits need to be considered clearly to obtain effective results of GWAS. In this study, the relative values were calculated to emphasize the changes in the seedling weight before and after the low-temperature treatments, which ensure that mapped QTLs were specifically involved in stress responses. Finally, a multi-locus model (FarmCPU) adopted by this study was based on multiple SNPs analysis, which could reduce false-positive sites by considering the linkage disequilibrium.

Finding causal variants and the candidate genes is challenging for GWAS since there are so many factors, such as linkage disequilibrium, false positive of the model, and phenotype error, which impact the ability to obtain precise results. RNA-seq analysis, haplotype analysis, and gene annotation could be used to partly solve the problems. For example, the gene LOC_Os01g62410 a R1R2R3 MYB gene have been studied and enhance cold tolerance by binding to the mitosis-specific activator cis-element in the promotor of OsCycB1;1, a G2/M phase-specific gene, and activating its expression [45]. In this study, we also integrated GWAS with the gene expression profiling during low-temperature stresses. The gene LOC_Os12g24490 is expressed in chilling acclimation, and there is phenotypic variation between different haplotypes and the gene LOC_Os12g22490, encoding C3HC4-type RING zinc finger protein function as a putative E3 ubiquitin-protein ligase, which is essential in the regulation of response to abiotic stress, and the cold-tolerance gene in
QTL ctb1 could interact with a subunit of the E3 ubiquitin ligase, SKP1, suggesting that a ubiquitin-proteasome pathway is involved in cold tolerance [45]. Previous studies also found that diverse environmental stresses (including cold stress) induced the expression of BrRZFP1 (C3HC4-type RING zinc finger protein) in Brassica rapa, and overexpression of BrRZFP1 conferred increased tolerance to cold, salt, and dehydration stresses [46]. Ectopic overexpression of a novel wheat zinc finger transcription factor TaZnF conferred heat-stress tolerance and cold and oxidative stresses in Arabidopsis [47]. In addition, a gene encoding C3HC4-type RING finger was linked to fruit quality and chilling injury in peach [48]. These results clearly confirmed the roles of candidate genes obtained in our study in the chilling acclimation in rice, and meanwhile, these findings imply the reliability of our GWAS results. It will be of importance to identify the mechanism of both genes in chilling acclimation in crops.

4. Materials and Methods

4.1. Phenotyping for GWAS

The 338 rice accessions analyzed were from the 3K Rice Genome Project. This fieldwork was conducted at the experiment station in Hainan in 2019. The experiment was conducted in a growth chamber under 12 h light/12 h dark conditions (26 °C and RH 55%). To screen for the cold stress and chilling acclimation exposure variability in the collected germplasm, shoot fresh weights under different conditions were used for GWAS. For chilling acclimation exposure, cold-stress treatments were performed in the growth chamber at the seedling stage (7-day-old), the rice seedlings were treated by 12 °C (day/night) for 2 days (cold stress), and then, the temperature was decreased to 6 °C (day/night) for 3 days as chilling-acclimation treatment; for cold stress, the seedlings were exposed to 6 °C (day/night) for 3 days then recovered for 9 days. For control, the seedlings continued to grow at 25 °C (day/night). Then, the shoot fresh weight was measured at same time after cold-stress treatment.

In addition, the calculation method of relative value referred to a previous study that used the relative changes in tiller numbers as the calculation of nitrogen response [9]. The relative shoot fresh weight under cold stress was calculated with the value of (shoot fresh weight under normal temperature—shoot fresh weight under cold stress)/shoot fresh weight under normal temperature, and this was used as an effective measurement of the response to cold stress. Similarly, the relative shoot fresh weight under chilling acclimation was calculated with the value of (shoot fresh weight under normal temperature—shoot fresh weight under chilling acclimation)/shoot fresh weight under normal temperature.

4.2. Genotyping for GWAS

Genetic variations of 338 accessions were downloaded from https://s3.amazonaws.com/3kricegenome/snpseek-dl/, accessed on 22 June 2022. SNPs were filtered with minor allele frequencies 0.05, missingness per marker 0.02, and missingness per individual 0.01. PLINK was used to deal with the raw data, the principle components (PCs), and kinship matrix analyses. The first two matrices of principle component analysis were used to construct the PC matrix. The LD of this panel was calculated by LD decay software (Version 3.41) [23]. The -log10(p) = 5 was used, and the LD heatmaps were constructed using LDBlockShow software (Version 1.40). The GWAS was performed using the FarmCPU model by rMVP [26]. ANNOVAR was used as to annotate the SNPs [49]. R and TBtools were used as data management and statistical analysis [50].

4.3. Integrated Analyses of GWAS and RNA-seq

To reduce the influence of negative positive SNPs, regions with two or more SNPs were selected, and then, regions around these SNPs were considered as QTLs. To find the candidate genes in the QTL, we performed seven RNA-seq (CK, 6 °C 6 h, 6 °C 24 h, 12 °C 6 h, 12 °C 24 h, 12 °C 24 h + 6 °C 6 h, and 12 °C 24 h + 6 °C 24 h) for the cold stresses and chilling acclimation in rice seedlings of Nipponbare (NPB). The sequencing was carried out
by BGI Illumina, and the detailed method of RNA-seq analyses and results can be found in our previous study [51]. Then, we integrated genes in QTLs with RNA-seq data, and genes with a higher expression were chosen for further study.

4.4. qRT-PCR Analysis of Two Candidate Genes

Seeds of rice variety Zhonghua 11 (ZH11) were surface-sterilized in 70% ethanol for 2 min followed by gentle shaking in the 2.5% NaClO for 30 min and then washed several times with sterilized water. Sterilized seeds were soaked in distilled water for 4 d and germinated in the incubator. After that, seedlings were grown hydroponically in a growth chamber under 12 h light/12 h dark condition at 25 °C for 14 d, with constant illumination intensity of about 500 µmol.m⁻².s⁻¹ and a relative humidity of 55%. Rice seedlings were then subjected to the different chilling stresses, and shoot tissues from six plants were collected at the designated time points, immediately frozen in liquid nitrogen, and stored at −80 °C until use. Total RNA was extracted from leaves of 2-week-old ZS11, and the primers for two candidate genes used are listed below: LOC_Os01g62410 F: 5′-GACAAGCGGGCCAATAAGGA-3′, LOC_Os01g62410 R: 5′-ATCGATGCAAGCATTGTACCTCT-3′; LOC_Os12g24490 F: 5′-TCGTCGTGCTCTACCTGTT-3′, LOC_Os12g24490 R: 5′-TACTCGAACGCCGGGAT-3′.

5. Conclusion

In this study, we focused on novel cold stress and chilling acclimation of rice seedlings. A total of 338 accessions of rice core germplasms was used, and relative shoot fresh weights at different temperature conditions were calculated for GWAS, and candidate genes within 12 QTLs were integrated with RNA-seq. Then, haplotype analysis was carried out for three selected genes. In summary, there is a shared pathway between cold stress and chilling acclimation, but most of the pathways were unique for chilling acclimation or cold stress. This study provides a platform to further analysis of chilling acclimation exposure in rice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms232113208/s1.

Author Contributions: J.L. performed the data analysis and wrote the manuscript; A.A.K. collected the shoot fresh weight data of rice population; L.H. and J.Y. participated in the database searches and data analyses; L.Z. performed the RNA extraction and qPCR experiment; L.W. and G.X. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The information of the 338 accessions of this study are openly available at https://snp-seek.irri.org, accessed on 22 June 2022.

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