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QTL Mapping of Mesocotyl Elongation and Confirmation of a QTL in Dongxiang Common Wild Rice in China

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Abstract: Direct-seeded rice (DSR) cultivation is an effective and important way to resolve agricultural labor scarcity, water scarcity and high production cost issues. Mesocotyl elongation (ME) is the main driver of the rapid emergence of rice seedlings from the soil and is an important indicator of the suitability of rice varieties for direct seeding. Hence, discovering ME-related genes is particularly important for breeding rice varieties suitable for direct seeding. In this study, a chromosome segment substitution line (CSSL) population generated from a cross between Dongxiang common wild rice and Nipponbare (Nip) was used to map quantitative trait loci (QTLs) for ME. Two QTLs for mesocotyl length were identified on chromosomes 3 and 6 with logarithm of odds (LOD) scores ranging from 5.47 to 6.04 and explaining 15.95–16.79% of the phenotypic variance. Among these QTLs, *qML6* accounted for the highest phenotypic variance (16.79%). Then, to confirm the strongest QTL, we generated an F_2 segregating population via the CSL127 line harboring the *qML6* locus and the recurrent parent Nip. The QTL *qML6-1* associated with ME was mapped to a location between markers DX-C6-2 and DX-C6-4, which is consistent with the location of the previously mapped QTL *qML6*. *qML6-1* had an LOD score of 8.45 and explained 30.56% of the phenotypic variance. The QTLs detected in this study provide promising targets for further genetic characterization and for use in marker-assisted selection to develop varieties with improved ME for the cultivation of DSR.

Keywords: direct-seeded rice; mesocotyl elongation; chromosome segment substitution line; wild rice

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1. Introduction

Direct-seeded rice (DSR) refers to rice seeds simply sown directly in the field, circumventing the steps of germination and transplanting. Compared with the traditional transplanting system, the DSR system has received increasing attention in rice growing regions around the world, as the DSR system is time saving, labor saving, and efficient [1–4]. According to the water conditions in the field at the time of sowing, DSR methods can be divided into dry direct seeding (sowing dry seeds onto dry soil), wet direct seeding (sowing pregerminated seeds onto wet, puddled soils), and waterlogged direct seeding (sowing seeds into standing water) [5]. The production of DSR generally faces three major problems, namely, difficulties involving seedling emergence, problems associated with severe grass damage and problems due to plant lodging. Difficulties associated with seedling emergence are the primary problems restricting the yield of DSR, which hinders the promotion of DSR on a large scale [6]. In the process of DSR cultivation, the elongation of the mesocotyl (the tissue between the insertion point of the radicle and the coleoptile node) is an important characteristic affecting the emergence of seedlings [7–9]. The elongation of the mesocotyl moves the germ and coleoptile close to the soil surface, allowing seedlings to grow in flooded and dark soil layers, thus ensuring high seedling survival and overall

emergence [10,11]. Germplasms with a short mesocotyl have insufficient soil-breaking ability, exhibit slow emergence, and have a low survival rate, while germplasms with a long mesocotyl have a fast emergence rate, a high survival rate, and uniform emergence [12]. However, due to the long-term use of transplanting during cultivation, the number of rice varieties with mesocotyl elongation (ME) used in current production is low. Discovering the genes controlling ME and revealing the mechanisms controlling ME are essential for improving ME characteristics and for breeding new rice varieties suitable for direct seeding.

Rice has a long history of domestication, and there is an extremely rich abundance of germplasm resources. The heritability of mesocotyl length is high, and the differences in ME characteristics among different rice germplasm resources are obvious. It has been reported that, in general, the mesocotyl of indica rice is longer than that of japonica rice, and that of weedy rice is longer than that of cultivated rice [13–16]. Due to the differences in ME characteristics among resources, combined with the use of molecular markers, quantitative trait locus (QTL) mapping for ME in rice has been carried out by several research groups. Using an F_2 population constructed with japonica Labelle and indica BlackGora, Redoña et al. [17] detected five QTLs affecting ME on chromosomes 1, 3, 5, and 7. Three QTLs controlling mesocotyl length were identified by the use of an $F_{2:3}$ population resulting from a cross between the Indian cultivar Surjamkhi and the Chinese cultivar Dao Ren Qiao [18]. Using a doubled haploid population from a cross between indica and japonica cultivars, Cao et al. [19] detected eight QTLs and found that ME is mainly affected by epistasis and additive effects. Ou-yang et al. [20] used a recombinant inbred line (RIL) population to map 27 QTLs for ME. Lee et al. [21,22] generated a backcross inbred line (BIL) population using Kasalath and Nipponbare (Nip) and conducted QTL mapping for ME in 2012 and 2017 via dark box and deep-seeding conditions, and five and three QTLs were identified, respectively. Huang et al. [10] and Niu et al. [23] used an RIL population to map five and three QTLs for ME, respectively. In addition to using linkage analysis to locate ME-related QTLs, several researchers have identified ME loci via genome-wide association analysis of natural populations. Wu et al. [24] detected nine ME loci in 170 core germplasms and 100 drought-tolerant materials; Lu et al. [25] detected 17 ME loci within 469 indica rice materials; and Liu et al. [26] detected a total of 16 ME loci in 208 rice materials under sand, soil, and hydroponic conditions. Jang et al. [27] used a core collection of 137 rice accessions and identified 11 QTLs for ME. As mentioned above, more than 100 QTLs for ME have been reported, and four QTLs, *qML1/qML1-1*, *qML3/qMel-3*, *qML6/qMel-6* and *qML7/qML7-1*, on chromosomes 1, 3, 6 and 7 have been identified several times. However, the vast majority of ME QTLs were detected in cultivars and landraces using temporary genetic populations, and there are few reports on QTL analysis involving wild rice resources. A chromosome segment substitution line (CSSL) population is generally developed through crossing, advanced backcrossing with marker-assisted selection and self-crossing. Each final CSSL carries a single or several chromosomal segments from the donor parent in the background of the recurrent parent and can thus be regarded as a near-isogenic line of the recurrent parent. The CSSL strategy is particularly suitable for the construction of a genetic population with wild rice as the donor parent. In addition, CSSLs can also minimize linkage drag, and facilitate the map-based cloning of QTLs [28]. Hence, genetic studies involving additional sources, such as wild rice, in genetic populations with high QTL detection efficiency and stability, are needed to discover novel QTLs.

In this study, to discover new QTLs or major QTLs for ME in wild rice, we used a set of CSSLs derived from Dongxiang common wild rice and Nip as materials to analyze QTLs. Our results will provide important information for further functional analysis of ME-related genes and the discovery of excellent alleles for DSR variety improvement.

2. Materials and Methods

2.1. Plant Materials

Previously, our laboratory used Dongxiang common wild rice as the donor parent and Nip as the recurrent parent to develop a set of 104 CSSLs through crossing and backcrossing with marker-assisted selection based on 203 polymorphic molecular markers. The 104 CSSLs covered 87.94% of the genome of Dongxiang common wild rice, and each CSSL contained an average of four introgressed segments [28]. In this study, we used this set of CSSLs as plant materials. All of the materials, including the CSSLs and the recurrent parent Nip, were planted in the field at the Institute of Crop Sciences of the Chinese Academy of Agricultural Sciences (CAAS), and the plants were managed in accordance with standard practices.

To confirm the largest QTL, *qML6*, we selected the line CSL127, which contains Dongxiang common wild rice chromosome segment harboring *qML6* locus, to cross with the recurrent parent Nip and generated an F_2 segregating population. CSL127 has exceeded the BC_5F_8 generations and is a stable pure line. For the convenience of description, we subsequently express this segregating population as F_2. A total of 119 F_2 individuals were used to develop $F_{2:3}$ families for collecting phenotypic data.

2.2. Evaluation of ME

The seeds were placed in an oven at 45 °C for 1 week to break dormancy. Fifty full and uniform seeds were selected, placed in 50 mL centrifuge tubes, and sterilized with 10% sodium hypochlorite. The seeds were then placed in an oven at 35 °C for 2 days for germination. Afterward, 30 seeds displaying relatively uniform radicle lengths were placed in 96-well germination plates in plastic pots (55 cm long, 40 cm wide and 10 cm high) with 2 L of pure water and then incubated at 28 °C for 7 days in the dark. ME length, which was measured as the distance from the root base to the coleoptile node, was measured for each seedling using a ruler. Seedlings with poor growth were removed.

2.3. Genotypic Data of the F_2 Segregating Population and QTL Analyses

The QTLs controlling ME in the CSSLs were mapped by using previously reported genotypic data from our laboratory [28,29]. The CSL (QTL mapping in CSS lines) functionality was used to identify QTLs by the likelihood ratio test based on the stepwise regression for additive QTL (RSTEP-LRT-ADD) mapping method, which was developed for QTL mapping in nonideal or ideal CSSL populations [30]. The largest *p*-value for entering variables in the stepwise regression of residual phenotype on marker variables (PIN) was set to 0.001, and the largest *p*-value for removing variables (Pout) was set to 0.002. Permutations of 1000 times tests were used to determine LOD thresholds to declare significant QTL ($p < 0.05$) and to calculate the genome-wide type I error for each QTL [31]. QTL detection by RSTEP-LRT, the calculation of phenotypic variation explained (PVE) by each QTL, and the estimation of QTL additive effects, were implemented by QTL IciMapping software [32].

To obtain genotypic data for the F_2 segregating population, young leaves from each plant were harvested, and genomic DNA was extracted using the conventional cetyltrimethylammonium bromide method [33]. Genotyping was performed using polymorphic InDel markers (Supplementary Table S1). Each PCR amplification was performed in a 10 μL mixture, which consisted of 50 ng of genomic DNA, 0.5 μL of each forward and reverse primer (10 $\mu mol \cdot L^{-1}$), 5 μL of 2 × Taq PCR Mix for polyacrylamide gel electrophoresis (PAGE) (Genesand Biotech, Beijing, China), and enough ddH_2O to reach the total reaction volume. The PCR conditions were as follows: 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 30 s, with a final extension at 72 °C for 5 min; and then holding at 16 °C. The PCR products were separated via electrophoresis on 8% polyacrylamide gels, and the results were observed and recorded using a gel imaging system. The linkage map was constructed using QTL IciMapping software with the Kosambi mapping function [32]. The anchor only was used in grouping, the ordering algorithm was anchor order, and the criterion used in rippling was the sum of adjacent recombination fractions (SARF) [32]. In

the F_2 segregating population, QTL analysis was conducted by the inclusive composite interval mapping for additive QTL (ICIM-ADD) mapping method [34]. The determination of the LOD threshold, QTL detection, PVE calculation, and additive effect estimation were performed with the same method as for the CSSL population described above.

3. Results

3.1. Phenotypic Variation

The phenotypic distribution of ME in Nip and the CSSLs is shown in Figure 1. The mean mesocotyl length for Nip was 3.9 mm. However, the mean mesocotyl length was 4.5 mm for the CSSLs, ranging from 1.7 to 10.9 mm, and the coefficient of variation was 49%. The mesocotyl length of the CSSLs presented a continuous distribution, indicating that it is a quantitative trait controlled by multiple genes in rice.

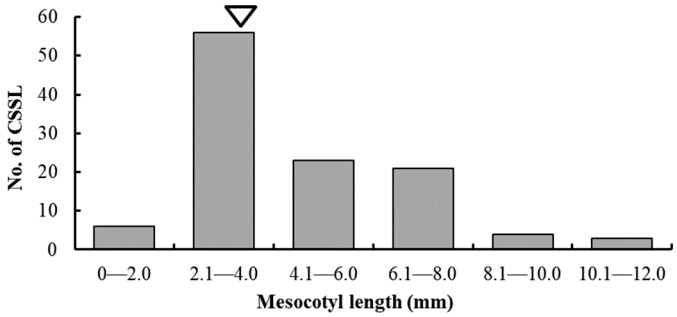

Figure 1. Frequency distribution of the mesocotyl length of the CSSLs. The triangle represents the mean mesocotyl length of Nip.

3.2. QTL Analysis of the ME

At the significant level of $p < 0.05$, the threshold LOD score of 3.34, was established using 1000 permutations in QTL IciMapping software [32]. Based on the analysis, two QTLs above the permutation threshold were identified in the CSSLs (Figure 2, Table 1), named *qML3* and *qML6*, with LOD scores of 5.47 to 6.04, explaining 15.95–16.79% of the phenotypic variation. *qML3* was located near the DX-C3-27 marker on chromosome 3; this QTL had an LOD score of 6.04, an additive effect of 9.5 mm and explained 15.95% of the phenotypic variation. *qML6* was located near the DX-C6-4 marker on chromosome 6; this QTL had an LOD score of 5.47, an additive effect of 4.1 mm, and explained 16.79% of the phenotypic variation. Dongxiang common wild rice alleles at all QTLs contributed to an increase in mesocotyl length.

3.3. Confirmation of the Largest QTL, qML6

According to the results of the QTL analysis presented above, the *qML6* locus explained the most phenotypic variation. Fortunately, we had a segregating population that could be directly used, and one of its parents was CSL127. CSL127 is an introgression line harboring Dongxiang common wild rice introgression at the *qML6* locus. Thus, we used this segregating population to perform a QTL analysis and check whether the locus could be repeatedly mapped.

A significant difference in mesocotyl length was found between the two parents, Nip and CSL127 (Figure 3). The mesocotyl of CSL127 was longer than 27.8 mm, whereas that of Nip was approximately 4.0 mm. The mean mesocotyl length was 11.6 mm among the plants in the F_2 segregating population, although the length ranged from 0 to 34.1 mm (Figure 4).

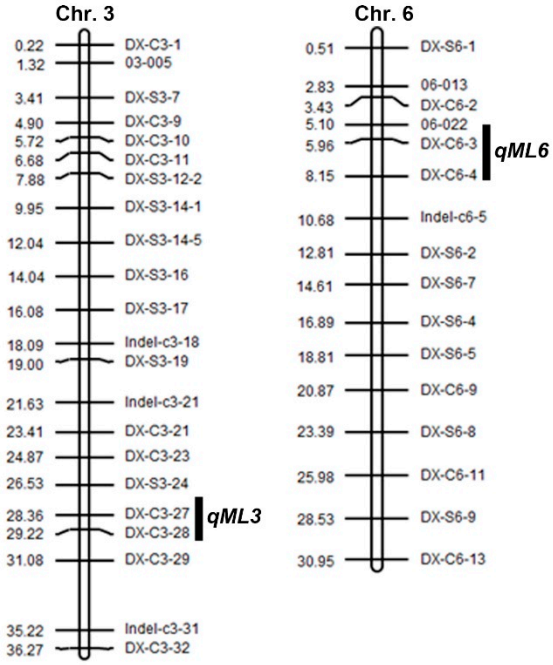

Figure 2. Chromosomal locations of QTLs for mesocotyl length in the CSSLs. The abbreviations for the QTLs are defined in Table 1. The position of each marker is based on the physical distance shown to the left of each chromosome, and the molecular marker is shown on the right. The positions of the two significant QTLs on chromosomes 3 and 6 are illustrated by solid black bars next to the chromosomes.

Table 1. QTLs for mesocotyl length detected in the CSSLs.

Locus	Chromosome	Marker Name	Position	LOD	PVE (%) [a]	Additive Effect [b]	Positive Allele [c]
qML3	3	DX-C3-27	28.36 Mb	6.04	15.95	9.5	DX
qML6	6	DX-C6-4	8.15 Mb	5.47	16.79	4.1	DX

[a] Phenotypic variation explained by individual QTLs. [b] Estimated effect of replacing Nip alleles with Dongxiang common wild rice. [c] DX represents the positive effects of QTLs contributed by Dongxiang common wild rice alleles.

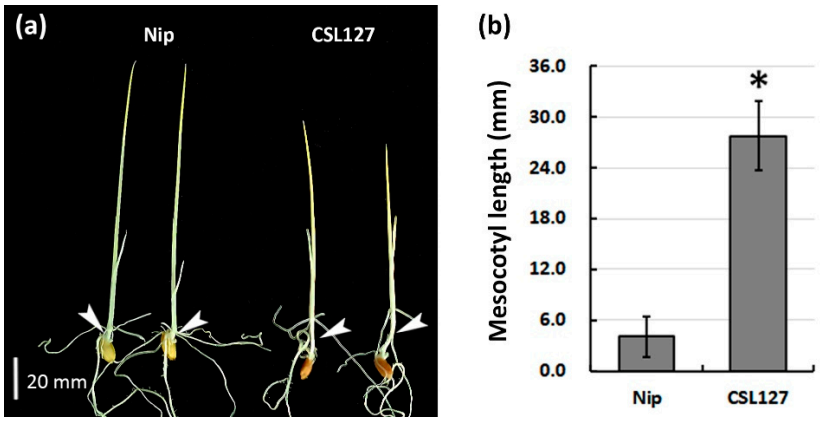

Figure 3. Comparison of ME between the Nip and CSL127 lines. (**a**) Seedlings of Nip and CSL127 after growing for 7 days in darkness. The arrows indicate the mesocotyls. Scale bar, 20 mm. (**b**) Mesocotyl length. The values are the means ± SD of 12 seedlings. The significance of differences between Nip and CSL127 was determined by Student's *t* test (*, $p < 0.01$).

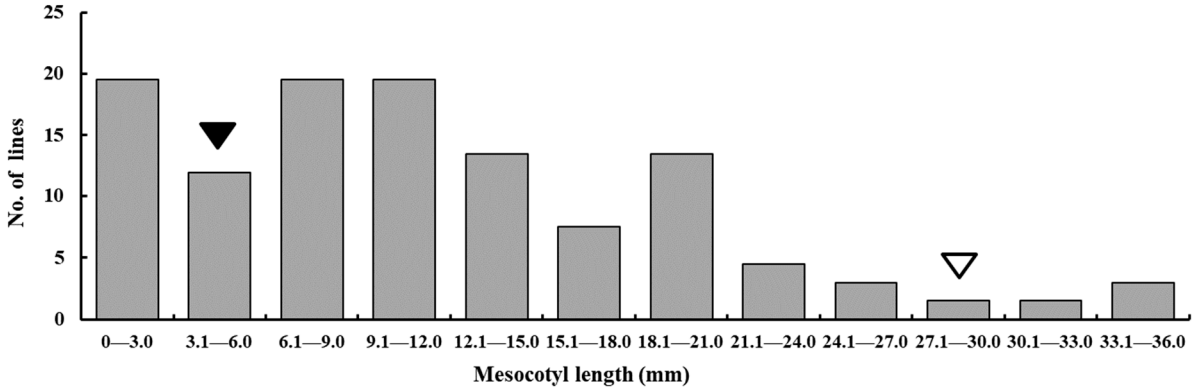

Figure 4. Frequency distribution of the mesocotyl length of plants within the F_2 segregating population. The black and white triangles represent the mean mesocotyl lengths of the Nip and CSL127 lines, respectively.

The CSL127 line contains six inserted segments from Dongxiang common wild rice on chromosomes 1, 2, 4, 6, 7 and 12. We genotyped each plant in the F_2 population via polymorphic indel markers in the six inserted segments. QTL analysis revealed that the LOD score of only one QTL, *qML6-1*, surpassed the threshold score of 3.61, which established using 1000 permutations ($p < 0.05$). No significant QTLs for mesocotyl length were detected, except for on chromosome 6 (data not shown). *qML6-1* was located between the markers DX-C6-2 and DX-C6-4 and had an LOD score of 8.45 and explained 30.56% of the phenotypic variation (Figure 5). The *qML6-1* and *qML6* positions overlapped, indicating that the *qML6* QTL is a reliable locus for ME.

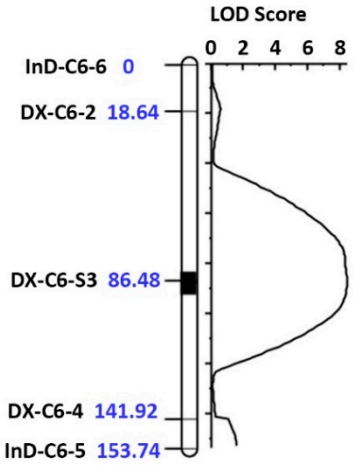

Figure 5. Confirmation of *qML6* via an F_2 segregating population. The position of the significant QTL, *qML6-1*, is illustrated by the solid black bar within the chromosome, and the LOD scores are shown on the right. On the left, the black text represents the molecular marker, and the blue text indicates the linkage position of the corresponding molecular marker on chromosome 6. The marker space unit is centimorgan.

4. Discussion

Rice (*Oryza sativa.* L) is one of the most important cereal crop species, as it provides food for more than half of the global population. However, during planting, germinating rice seeds, collecting the seedlings, and transplanting them necessitate considerable amounts of labor and material resources, and thus, DSR cultivation has gradually received increasing attention. However, due to the low seedling establishment rate and slow emergence of DSR, its application and promotion are limited. Elongation of the mesocotyl is the main driver of rapid seedling emergence in rice, and the vascular system of the seedling

mesocotyl transports nutrients and oxygen to the roots during the soil-breaking process to enable elongation under flooded conditions and within dark soil layers, thereby ensuring a higher emergence rate [35–38]. Therefore, ME is among the important breeding objectives with respect to the breeding of DSR varieties. In this study, we detected two QTLs on chromosomes 3 and 6. *qML6* and *qML3* were the two major QTLs, which explained 16.79% and 15.95% of the phenotypic variation, respectively.

Comparing the QTLs detected in this study with those previously reported, we found that *qML3* and *qML6* have been reported multiple times. The chromosomal position of *qML3* detected in this study is similar to that of *qMel-3*, *qml3*, and *qML3* reported by Lee et al. [22], Cao et al. [19], and Huang et al. [10], respectively. Similarly, in these three studies, *qMel-6*, *qml6* and *qML6* were found to be located at the same chromosomal position as *qML6* in the present study. In addition, Liu et al. [39] combined QTL sequencing (QTL-seq) and linkage analysis to identify the ME QTL *qML3* at the same location and speculated on the candidate genes in this region. Liu et al. [26] used genome-wide association analysis and identified a ME QTL within the 7.3–9.7 Mb region of chromosome 6, whose peak marker was rs_6_7313005; this QTL overlaps with the *qML6* locus in this study.

qML3 and *qML6* were the two major QTLs for ME that we mapped. The next step is to generate near-isogenic lines of these two loci and carry out fine mapping. Although we used CSSLs, several chromosomal segments of Dongxiang wild rice were inserted into the genome of many different lines. For example, the CSL66 line containing the *qML3* locus contains seven inserted segments, and the CSL127 line containing the *qML6* locus contains six inserted segments (data not shown). Therefore, it is not feasible to directly use the corresponding population of lines for fine mapping. In previous work, we crossed the CSL127 line with Nip and generated an F_2 segregating population. In the present study, we used this F_2 population to analyze QTLs for ME and verify that *qML6* is a positive-effect QTL. In future research, we can use this method to verify whether the mapped QTL has a positive effect to confirm the reliability of the results in advance and to improve the success rate of mapping genes underlying quantitative traits.

Although both ME-associated QTLs detected in this study have been reported previously, the allelic effects of these two loci differed significantly from those previously reported. This was particularly evident for the two main-effect QTLs *qML3* and *qML6*. In this study, *qML3* had the largest additive effect (9.5 mm), which is much larger than the 6.41 mm for *qMel-3* [22], 2.1 mm for *qml3* [19], and 2.9 mm for *qML3* [10]. *qML6* had a small additive effect of 4.1 mm, but this effect was also significantly greater than the 3.89 mm effect observed for *qMel-6* [22] and 1.7 mm observed for *qML6* [10]. These discrepant results may be related to the materials used in our study. We used a set of CSSLs, with Dongxiang common wild rice serving as the donor parent and Nip serving as the recurrent parent, to analyze QTLs for ME. Most previous studies have used RILs, BILs or natural populations generated from resources such as breeding varieties and landraces, and only one study used a temporary F_2 population derived from a cross of common wild rice W1944 and indica rice Pei-kuh [40]. Differences in the effects of the same QTL may be caused by differences in the study materials. Thus, the selection of appropriate or novel study materials is crucial for the discovery of new or large-effect QTLs. During the breeding process of varieties, many superior alleles in wild rice have been artificially selected. Therefore, research involving the use of wild rice resources can be used to identify alleles that have been lost from cultivated rice, apply these alleles to rice variety improvement, and broaden the genetic base of breeding parents.

5. Conclusions

As the number of farmers has declined, rice cultivation has undergone profound changes. DSR is becoming increasingly popular among farmers. The seedling establishment rate, one of the important factors affecting the yield of DSR, is closely related to ME. In this study, we used CSSLs generated from Dongxiang common wild rice and identified four QTLs for ME, of which *qML3* and *qML6* were the two main-effect loci. Compared with

the already reported ME-related loci, these two loci had larger additive effects. Moreover, using an existing F_2 segregating population, we verified that *qML6* was a reliable QTL. These QTLs should be subsequently finely mapped and cloned to understand the genetic control of ME to ensure seedling success and provide a useful genetic resource for DSR variety breeding.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12081800/s1, Table S1: Sequences of the primers used in this study.

Author Contributions: Q.H., X.M. and L.H. conceived and designed the study. Q.H., C.J., Y.C., D.C. and B.H. performed the experiments. Q.H. and X.M. analyzed all experiments data. Q.H., X.M. and Z.Z. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Not applicable.

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

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