Dynamic Quantitative Trait Loci Analysis of Seed Reserve Utilization during Three Germination Stages in Rice

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Abstract

In this study, one rice population of recombinant inbred lines (RILs) was used to determine the genetic characteristics of seed reserve utilization during the early (day 6), middle (day 10) and late (day 14) germination stages. The seedling dry weight (SDW) and weight of the mobilized seed reserve (WMSR) were increased, while the seed reserve utilization efficiency (SRUE) decreased, during the process of seed germination. The SDW and WMSR were affected by the seed weight, while the SRUE was not affected by the seed weight. A total of twenty unconditional and twenty-one conditional additive QTLs and eight epistatic QTLs were identified at three germination stages, and the more QTLs were expressed at the late germination stage. Among them, twelve additive and three epistatic QTLs for SDW, eight additive and three epistatic QTLs for WMSR and thirteen additive and two epistatic QTLs for SRUE were identified, respectively. The phenotypic variation explained by each additive QTL, epistatic QTL and QTL x development interaction ranged from 6.10 to 23.91%, 1.79 to 6.88% and 0.22 to 2.86%, respectively. Two major additive QTLs qWMSR7.1 and qSRUE4.3 were identified, and each QTL could explain more than 20% of the total phenotypic variance. By comparing the chromosomal positions of these additive QTLs with those previously identified, eleven QTLs might represent novel genes. The best four cross combinations of each trait for the development of RIL populations were selected. The selected RILs and the identified QTLs might be applicable to improve rice seed reserve utilization by the marker-assisted selection approach.

Introduction

Vigorous seedling growth is essential for profitable and sustainable crop production. Seedling establishment begins with the appearance of the radicle and terminates when the seedling has exhausted the seed’s energy reserves and starts to carry out photosynthesis [1]. Germination and heterotrophic growth are therefore crucial steps for the establishment of crops. By definition, seed vigor is the ability of a seed lot to establish seedlings successfully under a wide range of conditions [2]. High seed vigor, i.e., the rapid, uniform and complete emergence of vigorous seedlings, leads to high competition against weeds, uniform harvest and a high grain yield potential in rice. Improving rice seed vigor is an important objective in rice breeding programs.

Seed vigor is a complex trait that is determined by the interactions between genetic and environmental factors [3]. To date, several technological solutions, such as seed selection and seed treatment, have been used to improve seed vigor [2,4–7]. In recent years, the emphasis on improving seed vigor has shifted to the potential of genetic improvement in breeding programs. Quantitative trait loci (QTL) analysis for seed vigor had been reported in rice, tomato and lettuce [8–13]. Those QTLs can be used to improve seed vigor using a marker-assisted selection (MAS) breeding program; furthermore, it becomes possible to identify candidate genes associated with seed vigor [14].

As seeds imbibe water, the mobilization of seed reserves such as starch, proteins, and lipids from storage tissues or the endosperm begins, providing essential energy to fuel germination and seedling growth until the seedlings becomes photoautotrophic [15]. Heterotrophic seedling growth (seedling dry weight; SDW) can be defined as a product of two key traits: the weight of the mobilized seed reserve (WMSR) and the conversion efficiency of the utilized seed reserve (seed reserve utilization efficiency; SRUE) into seedling tissue [16–17]. These traits of seed reserve utilization play important roles in high seed vigor to improve germination and heterotrophic growth. Several phenotypic studies of seed reserve utilization have been conducted in wheat [16], soybean [17] and chickpea [18], but no studies have been reported in rice. Furthermore, the genetic control of seed reserve utilization in rice remains uncharacterized.

In rice, QTLs for seed vigor traits such as seed germination, seedling growth, seed longevity and tolerance to stress have been identified [8,11–15,19–25]. In most of these reports, the phenotypic values of the final developmental stage were used for QTL analysis. These QTLs with unconditional genetic effects (defined by Sen and Churchill [26]) are effective throughout seed

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germination. According to the theory of developmental genetics, genes are expressed selectively at different germination stages. QTL analysis can be adapted to include the effects of developmental stages [27]. Therefore, the QTL with conditional genetic effects (defined by Sen and Churchill [26]) are the loci detected at a specific growth stage [28]. The distinct gene actions for seed vigor at different developmental stages have been ignored in previous studies. Identification of both conditional and unconditional QTL will be desirable for MAS [28]. It will be essential, therefore, to include the dynamics of gene expression when analyzing the traits of seed reserve utilization.

Recently, several reports have explored the developmental behavior of various quantitative traits in rice [29–30], maize [31], cotton [32], soybean [28] and potato [33]. In rice, the dynamic QTL analysis has been used to dissect the genetic control of several quantitative traits such as tiller number [29–30], grain-filling [34] and root morphological traits [35]. The objectives of the present study were to investigate the developmental behavior of seed reserve utilization in rice, to identify both conditional and unconditional QTL and to measure the epistasis effects on seed reserve utilization during seed germination.

Materials and Methods

Plant materials

Two rice varieties, Jiucaiqing (japonica) and IR26 (indica), and 150 recombinant inbred lines (RIL) (F2:9) derived from Jiucaiqing × IR26 were used in this study [19–20]. All materials were grown at the Jiangpu Experimental Station of Nanjing Agricultural University, and the seeds were harvested at the mature stage. After the release of seed dormancy by post-ripening after four months of storage at room temperature (22–26°C), the seeds were used for the germination test.

Evaluation of seed reserve utilization

To determine the seed water content (WC) of each RIL and their parents, three replicates of seed (~4.0 g per replicate) were powdered and dried at 104°C for 24 h. For the evaluation of seed reserve utilization, three replicates of each RIL and their parents (thirty seeds per replicate) were used and weighed (W). According to the corresponding WC, the initial seed dry weight (W0) (ISDW; mg per seed) of each RIL and their parents were calculated as [W × (1–WC)/30].

Then, the seed germination was conducted in a growth chamber at 30±1°C in the dark for 6, 10 or 14 days according to the methods of Zhang et al. [24]. The shoots and roots of seedlings were measured from seeds by hand after germination. The seedling dry weight (W1) (SDW; mg per seed) and the remnant seed dry weight (W2) (RSDW; mg per seed) were obtained after oven drying at 104°C for 24 h. The weight of the mobilized seed reserve (W3) (WMSR; mg per seed) was calculated as [W0–W2]. The seed reserve utilization efficiency (SRUE; mg per mg) was calculated as [W1/W3] [16].

Data analysis

Experimental data were analyzed using the Statistical Analysis System (SAS) software, and the traits of the parents were compared using Student’s t-test at the 5% and 1% levels of probability. The correlations of the traits were computed using the PROC CORR by SAS software [19–20].

QTL mapping

The linkage map has been constructed previously [19–20]. Finally, a set of 168 SSR markers covering most of the rice genome at an average interval of 15 cM was constructed. Unconditional QTL was detected using the mean phenotypic values at day 6, 10 and 14 of germination. The genetic behavior measured at time t is the result of genes expressed before time (t-1) and extra effects within the period from (t-1) to t. Conditional phenotypic values (t/t-L) at time t were given by subtracting the phenotypic means at time t-L from the mean at time t [27,36]. Conditional QTL was detected using the mean phenotypic values (t/t-L) in the intervals from day 0 to 6 (t6/t0), 6 to 10 (t10/t6) and 10 to 14 (t14/t10) of germination.

Using the phenotypic values at a single germination stage, the unconditional and conditional additive QTL, was identified respectively by the inclusive composite interval mapping (ICIM) method in QTL IciMapping [37–38]. Briefly, the P values for entering variables (PIN) and removing variables (POUT) were set at 0.01 and 0.02, and the scanning step was 1 cM [19]. The LOD score of 2.5 was used as the threshold value to declare the presence of a putative additive QTL. The QTNetwork program ver. 2.0, based on a mixed linear model [39] was used to identify the epistatic QTL for seed reserve utilization in joint analysis of the unconditional phenotypic values of three germination stages. Briefly, the values for testing window and filtration window were set at 10 cM, respectively, and the walking speed was 1 cM. The putative QTL detection was determined at 95% confidence level. The proportion of observed phenotypic variance explained by each additive and epistatic QTL and the corresponding additive effects were also estimated. The QTL nomenclature followed the method of McCouch and CGSNL [40].

Prediction for novel parental combination

The best cultivars with maximum phenotypic value and elite alleles might be used to design parental combinations for crop breeding [41]. According to the information of phenotypic values and alleles of detected loci among the RILs, the best four cross combinations of each trait were selected. Firstly, the RILs with relatively highest phenotypic values of SDW, WMSR or SRUE at three germination stages (Day 6, 10 and 14) were selected. Then, the positive alleles of the detected additive QTLs among the selected RILs were analyzed. Using Monte Carlo simulation experiments, a novel cultivar combination can be determined by maximizing the number of elite alleles [41]. Finally, the best four cross combinations of each trait were selected.

Results

Seed reserve utilization phenotypes

During the process of seed germination, the SDW and WMSR were increased among the parents and RILs, while the SRUE was decreased. There were significant differences in the SDW, WMSR and SRUE between two parents at the middle (Day 10) and late (Day 14) germination stages (Table 1). The SDW and WMSR of Jiucaiqing were significantly higher than those of IR26 at the middle and late germination stages. In contrast, IR26 had a significantly higher SRUE at the middle and late germination stages. There was a continuous frequency distribution and transgressive segregation in the SDW, WMSR and SRUE among RIL population at three germination stages.

Correlation of seed weight with seed reserve utilization related traits

There were significant positive correlations between the ISDW and SDW during three germination stages (Table 2). The correlations between the ISDW and the WMSR were significantly positive during the middle and late germination stages, but there...
were no significant correlations between ISDW and SRUE. These results indicated that the SDW and WMSR are affected by the seed dry weight, while not the SRUE.

Unconditional and conditional QTL for SDW

A total of six unconditional additive QTLs were identified for the SDW at three germination stages (Table 3; Fig. 1). One QTL (qSDW1.2) and six QTLs (qSDW1.1, qSDW2, qSDW4.2, qSDW5, qSDW8.2 and qSDW9) were detected at day 10 and 14 stage, respectively. Among them, the qSDW1.2 was consistently detected in both germination stages. The phenotypic variance explained by each QTL ranged from 6.78 to 16.75%. The positive alleles of three QTLs (qSDW1.2, qSDW4.2 and qSDW9) from Jiucaiqing and four QTLs (qSDW1.1, qSDW4.2 and qSDW8.2) from IR26 contributed to the increase of SDW.

Seven conditional additive QTLs were identified for the SDW during three germination stages (Table 3; Fig. 1). One QTL (qSDW1.2) and six QTLs (qSDW1.1, qSDW3, qSDW4.1, qSDW8.1, qSDW8.2 and qSDW8.3) were detected during day 6 to 10 stage and day 10 to 14 stage, respectively. The phenotypic variance explained by each QTL ranged from 6.97 to 16.18%. The positive alleles of three QTLs (qSDW1.1, qSDW3 and qSDW8.1) from Jiucaiqing and four QTLs (qSDW4.1, qSDW8.2, qSDW8.3 and qSDW12) from IR26 contributed to the increase of SDW.

Unconditional and conditional QTL for WMSR

Four unconditional additive QTLs were identified for the WMSR at three germination stages (Table 3; Fig. 1). Two QTLs (qWMSR2 and qWMSR6), one QTL (qWMSR7.1) and two QTLs (qWMSR7.2 and qWMSR10) were detected at day 6, 10 and 14 stage, respectively. Among them, the qWMSR4 was consistently detected in both day 6 and 14 stages. The phenotypic variance explained by each QTL ranged from 8.40 to 14.60%. The positive alleles of one QTL (qWMSR3) from Jiucaiqing and three QTLs (qWMSR1, qWMSR2 and qWMSR6) from IR26 contributed to the increase of WMSR.

Seven conditional additive QTLs were identified for the WMSR during three germination stages (Table 3; Fig. 1). Two QTLs (qWMSR2 and qWMSR6) and five QTLs (qWMSR1, qWMSR7.1, qWMSR7.2, qWMSR10 and qWMSR12) were detected during day 0 to 2 stage and day 6 to 10 stage, respectively. The phenotypic variance explained by each QTL ranged from 8.04 to 20.51%; the major QTL (qWMSR7.1) accounted for the largest amount of phenotypic variation (20.51%) during the day 6 to 10 stage. The positive alleles of two QTLs (qWMSR7.1 and qWMSR10) from Jiucaiqing and five QTLs (qWMSR1, qWMSR2, qWMSR4, qWMSR7.2 and qWMSR12) from IR26 contributed to the increase of WMSR.

Table 1. Phenotypic values of seed reserve utilization related traits among the parents and RILs population at three germination stages.

| Germination stages (d) | Traits* | Parentsb | RIL Populationc |
|------------------------|---------|----------|-----------------|
|                        |         | Jiucaiqing | IR26 Mean | Max | Min | SD | Skewness | Kurtosis |
| 6                      | SDW     | 2.81±0.14 | 2.54±0.37 | 3.73 | 5.96 | 1.41 | 0.88 | −0.06 | −0.04 |
|                        | WMSR    | 3.50±0.49 | 3.33±0.26 | 4.33 | 8.25 | 1.74 | 1.21 | 0.48 | 0.28 |
|                        | SRUE    | 0.80±0.17 | 0.76±0.09 | 0.86 | 0.99 | 0.33 | 0.11 | −1.57 | 3.78 |
| 10                     | SDW     | 4.55±1.02** | 3.71±0.81 | 5.74 | 9.77 | 2.61 | 1.29 | 0.68 | 1.03 |
|                        | WMSR    | 10.52±0.27** | 4.88±0.60 | 8.85 | 15.95 | 4.40 | 1.90 | 0.93 | 1.90 |
|                        | SRUE    | 0.43±0.04 | 0.76±0.02** | 0.65 | 0.80 | 0.48 | 0.06 | −0.16 | 0.04 |
| 14                     | SDW     | 6.29±0.85** | 5.43±1.49 | 6.93 | 11.13 | 2.53 | 1.32 | 0.12 | 0.57 |
|                        | WMSR    | 11.44±0.48** | 8.43±1.15 | 10.79 | 16.28 | 5.09 | 1.84 | 0.13 | 0.40 |
|                        | SRUE    | 0.55±0.03 | 0.64±0.13** | 0.64 | 0.94 | 0.32 | 0.07 | −0.58 | 5.63 |

*SDW: seedling dry weight, mg per seed; WMSR: weight of mobilized seed reserve, mg per seed; SRUE: seed reserve utilization efficiency, mg per mg.  
**indicates significance at the level of 1% according to Student’s t-test.

Table 2. Correlation coefficients between seed reserve utilization related traits and seed weight at three germination stages.

| Traits* (Day 6) | ISDW | Traits (Day 10) | ISDW | Traits (Day 14) | ISDW |
|----------------|------|----------------|------|----------------|------|
|                | t0   | t6/t0          |      | t10/t6         |      |
| SDW            | 0.312** | 0.312** | SDW   | 0.601** | 0.379** | SDW   | 0.683** | 0.259** |
| WMSR           | −0.187 | −0.187 | WMSR  | 0.606** | 0.488** | WMSR  | 0.736** | 0.249** |
| SRUE           | 0.073 | 0.073 | SRUE  | 0.113 | 0.051 | SRUE  | 0.086 | −0.089 |

*ISDW: initial seed dry weight; SDW: seedling dry weight; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency.  
**Unconditional phenotypic values at the day 6, 10 and 14 of germination.  
†Conditional phenotypic values in the intervals from day 0 to 6 (t0/t0), 6 to 10 (t6/t0) and 10 to 14 (t10/t6) of germination;  
Indicates significance at the level of 1%.  
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### Table 3. Unconditional and conditional QTLs for seed reserve utilization related traits at three germination stages.

| Traits   | Germination stage (d) | t<sup>1</sup> | LOD | \( r^2 \) (%)<sup>2</sup> | A<sup>3</sup> |
|----------|-----------------------|--------------|-----|----------------|--------|
|          | QTLs                  | Chr.<sup>4</sup> Marker interval |     |                |        |
|          |                       |               |     |                |        |
| QTLs     |                       |               |     |                |        |
| SDW      | 10                    | qSDW1.2       | 1 RM6950–RM5759 | 3.96  | 12.93 | -0.54 |
|          | 14                    | qSDW1.2       | 1 RM6950–RM5759 | 3.41  | 10.92 | -0.51 |
|          |                       | qSDW2         | 2 RM5651–RM5804 | 3.16  | 6.78  | 0.35  |
|          |                       | qSDW4         | 4 RM273–RM3839 | 7.06  | 16.75 | -0.54 |
|          |                       | qSDW5         | 5 RM159–RM3702 | 2.97  | 8.29  | 0.38  |
|          |                       | qSDW8         | 8 RM3342–RM3459 | 4.43  | 13.46 | -0.54 |
|          |                       | qSDW9         | 9 RM6570–RM3164 | 3.07  | 7.09  | 0.36  |
| WMSR     | 6                     | qWMSR2        | 2 RM240–RM5404 | 4.82  | 13.44 | -0.48 |
|          |                       | qWMSR4        | 4 RM3389–RM3367 | 2.67  | 8.40  | -0.36 |
|          |                       | qWMSR1        | 1 RM6950–RM5759 | 4.80  | 14.60 | -0.84 |
|          |                       | qWMSR3        | 3 RM282–RM6800 | 2.57  | 9.04  | 0.57  |
|          |                       | qWMSR4        | 4 RM3389–RM3367 | 3.60  | 10.20 | -0.59 |
| SRUE     | 6                     | qSRUE2.1      | 2 RM5631–RM3685 | 4.03  | 11.02 | -0.04 |
|          |                       | qSRUE2.2      | 2 RM240–RM5404 | 7.12  | 18.65 | 0.05  |
|          |                       | qSRUE4.2      | 4 RM3839–RM3367 | 2.67  | 8.40  | -0.36 |
|          |                       | qSRUE4.3      | 4 RM15626–RM5687 | 8.91  | 23.91 | -0.03 |
|          |                       | qSRUE12.2     | 12 RM5609–RM6953 | 3.01  | 8.22  | -0.03 |
|          |                       | qSRUE4.1      | 4 RM335–RM518 | 3.66  | 9.54  | 0.02  |
|          |                       | qSRUE4.2      | 4 RM518–RM16535 | 3.22  | 7.67  | 0.02  |
|          |                       | qSRUE4.3      | 4 RM15626–RM5687 | 8.91  | 23.91 | -0.03 |
|          |                       | qSRUE12.2     | 12 RM5609–RM6953 | 3.15  | 10.79 | -0.01 |
|          |                       | qSRUE4.4      | 4 RM7187–RM3687 | 4.15  | 14.59 | -0.03 |
|          |                       | qSRUE4.5      | 4 RM3306–RM8220 | 3.36  | 9.22  | 0.02  |
|          |                       | qSRUE8.1      | 8 RM7027–RM6215 | 2.58  | 6.69  | 0.02  |

<sup>1</sup>SDW: seedling dry weight; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency;
<sup>2</sup>Unconditional QTL at the day 6, 10 and 14 of germination;
<sup>3</sup>Conditional QTL in the intervals from day 0 to 6 (t<sub>t-1</sub>), 6 to 10 (t<sub>t</sub>) and 10 to 14 (t<sub>t</sub>/t<sub>t+1</sub>) of germination;
<sup>4</sup>Chromosome on which the QTL was located;
<sup>5</sup>Variation explained by each putative QTL;
<sup>6</sup>Additive effect is the effect of substituting a Jiucaiqing allele for an IR26 allele; Its positive value indicates that Jiucaiqing has the positive allele; The case of negative values is just the opposite.

### Unconditional and conditional QTL for SRUE

Ten unconditional additive QTLs were identified for the SRUE at three different germination stages (Table 3; Fig. 1). Four QTLs (qSRUE2.1, qSRUE2.2, qSRUE4.2, and qSRUE12.2), four QTLs (qSRUE4.1, qSRUE4.2, qSRUE4.3 and qSRUE12.2) and three QTLs (qSRUE4.4, qSRUE4.5 and qSRUE8.1) were detected at day 6, 10 and 14 stage, respectively. Among them, the qSRUE12.2 was consistently detected in both day 6 and 10 stages. The phenotypic variance explained by each QTL ranged from 6.69 to 23.91%; the major QTL qSRUE4.3 accounted for the largest amount of phenotypic variation (23.91%) at the day 10 stage. The positive alleles of five QTLs (qSRUE2.2, qSRUE4.1, qSRUE4.2, qSRUE4.5 and qSRUE8.1) from Jiucaiqing and five QTLs (qSRUE2.1, qSRUE4.3, qSRUE4.4, qSRUE8.2 and qSRUE12.2) from IR26 contributed to the increase of SRUE.

Seven conditional additive QTLs were identified for the SRUE during three germination stages (Table 3; Fig. 1). Four QTLs (qSRUE2.1, qSRUE2.2, qSRUE8.2 and qSRUE12.2) and three QTLs (qSRUE1, qSRUE3 and qSRUE12.1) from Jiucaiqing and four QTLs (qSRUE1, qSRUE2.1, qSRUE8.2 and qSRUE12.2) from IR26 contributed to the increase of SRUE.

### Epistatic QTL for seed reserve utilization

A total of eight epistatic QTLs were identified by the joint analysis of the unconditional phenotypic values at three germination stages (Day 6, 10 and 14) (Table 4; Fig. 2). Among them, three epistatic QTLs each for SDW and WMSR were identified, respectively, with an epistatic main effect. The SRUE was controlled by two epistatic QTLs: one with both an epistatic main effect and an epistasis X development interaction effect and
one with only an epistatic main effect. The phenotypic variance explained by each epistatic QTL ranged from 1.79 to 6.88%, and the phenotypic variation explained by each epistatic QTL x development interaction ranged from 0.22 to 2.86%.

Prediction for novel parental combination
The best four cross combinations of each trait for the development of RIL populations were selected to improve seed reserve utilization (Table 5). Four RILs each for SDW, WMSR and SRUE were selected with a relatively high value at three germination stages respectively (Fig. 3). The selected RILs had two to eight positive alleles of the additive QTLs for each trait (Table 6). To improve the three traits (SDW, WMSR and SRUE) simultaneously, 33 elite alleles could be pyramided by the combination of RILs 1750, 1791, 1878, and 1890 (Table 6).

Discussion
Seed germination is shortly followed by the mobilization of seed reserves from the seed storage organs or endosperm, providing essential energy to fuel growth until the seedling becomes photoautotrophic [15]. Because of its importance in seed germination and post-germinative growth, the mobilization of reserves such as starch, protein and lipids during germination has been extensively studied [15,42–44]. In this study, we focused on the three traits (SDW, WMSR and SRUE) related to seed reserve utilization. The seedling establishment of the most RILs begins at day 6 of germination with the appearance of the radicle and shoot and the seed's energy reserves have mostly been exhausted at day 14 of germination. Therefore, the traits of seed reserve utilization were conducted at three germination stages (Day 6, 10 and 14). Those traits were identified in the dark to avoid the photosynthesis for energy usage in seed germination.
During seed germination, the dry weight of the growing seedling is always lower than that of the mobilized reserves due to respiration [16]. Similar results were observed in our results. Therefore, except for the WMSR, the SRUE, as the conversion efficiency of the mobilized reserve into seedling tissue, might be an important factor influencing vigorous seedling growth. In this study, we found that there were significant cultivar differences for seed reserve utilization that are in agreement with the findings of Soltani et al. [45] in wheat. The japonica variety Jiucaiqing had the higher WMSR, while the indica variety IR26 had the higher SRUE.

**Table 4.** The epistatic QTL for seed reserve utilization related traits in joint analysis of three germination stages.

| Traits   | Loci (i)b Chr. | Intervals | Loci (j)b Chr. | Intervals | A² (AD)c | AD¹ | AD² | AD³ | r² (A) (%)d | r² (AD) (%)d |
|----------|----------------|-----------|----------------|-----------|-----------|-----|-----|-----|-------------|-------------|
| SDW 1    | RM7341–RM7056  | 12        | RM6296–RM20    | 0.58**    | −0.12     | 0.02 | 0.10 | 6.16 | 0.73        |
| 2        | RM3685–RM5631  | 4         | RM7187–RM525   | 0.32**    | −0.16     | 0.03 | 0.13 | 4.50 | 1.18        |
| 5        | RM421–RM5970   | 6         | RM276–RM7420   | −0.45**   | 0.01      | −0.01| 0.01 | 5.30 | 0.22        |
| WMSR 1   | RM7341–RM7056  | 3         | RM3684–RM3564  | 0.48**    | −0.15     | 0.12 | 0.04 | 5.54 | 0.93        |
| 6        | RM340–RM1370   | 6         | RM276–RM7420   | −0.45**   | 0.12      | −0.08| −0.04| 4.42 | 0.71        |
| 7        | RM172–RM1306   | 9         | RM7048–RM7039  | 0.52**    | −0.07     | 0.02 | 0.05 | 6.88 | 0.68        |
| SRUE 7   | RM1132–RM5623  | 8         | RM6845–RM6976  | 0.01**    | 0.02**    | −0.01| −0.01| 1.79 | 2.86        |
| 8        | RM342–RM6215   | 12        | RM6953–RM5609  | 0.02      | 0.01      | −0.01| −0.01| 3.53 | 1.07        |

SDW: seedling dry weight; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency; aChromosome on which the QTL was located; bA represents the estimated additive effect of epistatic QTL, and AD1, AD2 and AD3 represents the additive effects of epistatic QTL at day 6, 10 and 14 of germination, respectively; Its positive value indicates that two loci genotypes being the same as those in parent Jiucaiqing (or IR26) take the positive effects, while the two-loci recombinants take the negative effects; c r² (A) and r² (AD) represents the phenotypic variation explained by the epistatic QTL and epistatic QTL × development interactions, respectively; d**indicates significance at the level of 1%.

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**Figure 2.** Location of epistatic QTLs for seed reserve utilization related traits on linkage groups in joint analysis of three germination stages. SDW: seedling dry weight; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency. The chromosome names are listed on the top left of the bar; the map distances (cM) are shown below the bar and the markers are shown above the bar.

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in the middle and late germination stages. Among the RIL population, the genetic difference in the SDW, WMSR and SRUE were also observed. These results indicate that genetic variation can be used in rice breeding programs for the improvement of seed reserve utilization.

In this study, we found that large seeds have the advantage of producing more vigorous seedlings, similar to previous results [16,46–47]. The ISDW was significantly corrected with the SDW and WMSR, while no significant relationship between ISDW and SRUE. Due to the SDW affected not only the WMSR but also the SRUE in germinating seed, the SDW of small seed with higher SRUE might be similar that of larger seed with low SRUE. Therefore, the lack of a significant positive relation between seed weight and seedling dry weight has also been reported in several studies [48–50].

Improving the trait of rice seed reserve utilization using conventional strategies is extremely difficult because it is a quantitative trait affected by both genetic and environmental factors. It is therefore necessary to perform QTL analysis of seed reserve utilization for marker assisted selection. Unconditional QTL mapping can explain cumulative gene actions from the beginning to the end of a specific process, and conditional mapping can identify the QTLs that act during a specific growth period but are not affected by the genes expressed in the previous stages [36]. In this study, a total of 20 unconditional QTLs were identified. Among them, 6, 6 and 11 unconditional QTLs were identified at day 6, 10 and 14 of germination stages, respectively. Similarly, a total of 21 conditional QTLs were identified. Among them, 6, 6 and 9 conditional QTLs were identified during day 0 to 6, 6 to 10 and 10 to 14 germination stages, respectively. These results showed that more QTLs were expressed during the late stage than during the early and middle stages. Combined using the conditional and unconditional QTLs, we can improve the seed reserve utilization traits not only during a specific germination period but also at the end of a specific germination stage by MAS.

We found that the additive QTLs were rarely co-localized among the three germination stages. Only the $q_{SDW1.2}$ was expressed at both day 10 and 14 germination stages, the $q_{WMSR4}$ was expressed at both day 6 and 14 germination stages, and the $q_{SRUE12.2}$ was expressed at both day 6 and 10 germination stages. These co-located QTLs might play important roles in controlling the seed reserve utilization during different germination stages. For the three traits of seed reserve utilization, the identification of co-localized additive QTLs only occurred in the genomic region RM6950–RM5759 of chromosome 1 for $q_{SDW1.2}$ and $q_{WMSR1}$, the genomic region RM240–RM5404 of chromosome 2 for $q_{WMSR2}$ and $q_{SRUE2.2}$ and the genomic region RM519–RM7376 of chromosome 12 for $q_{SDW1.2}$ and $q_{WMSR12}$. These co-localized QTLs could be very useful in the simultaneous improvement of more than one trait, as the desirable alleles of these additive QTLs (except for $q_{SRUE2.2}$) were

### Table 5. Parental combinations predicted from RILs of seed reserve utilization related traits.

| Trait  | Parental combination | Trait  | Parental combination | Trait  | Parental combination |
|--------|----------------------|--------|----------------------|--------|----------------------|
| SDW    | RIL 1702 × RIL 1791  | WMSR   | RIL 1750 × RIL 1774  | SRUE   | RIL 1814 × RIL 1824  |
|        | RIL 1702 × RIL 1890  |        | RIL 1750 × RIL 1878  |        | RIL 1814 × RIL 1829  |
|        | RIL 1750 × RIL 1890  |        | RIL 1774 × RIL 1905  |        | RIL 1814 × RIL 1890  |
|        | RIL 1791 × RIL 1890  |        | RIL 1878 × RIL 1905  |        | RIL 1829 × RIL 1890  |

*aSDW: seedling dry weight; WMSR: weight of mobilized seed reserve; SRUE: seed reserve utilization efficiency.

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Figure 3. The phenotypes of seed reserve utilization related traits among the selected RILs and two parents at three germination stages.

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contributed by a single parent, IR26. Additionally, the joint analysis of the multi-development phenotypic values suggested that epistatic QTLs and QTL x development interactions were important components for seed reserve utilization, even though the degree of interaction was low.

By comparing the chromosomal positions of these additive QTLs, 36 QTLs identified in previous studies were found to map near the additive QTLs identified in this study (Fig. 4). The qSDW1.2 and qWMSR1 located to a position that coincided with the region of qSH1.2 for shoot height [20], and the qSDW1.1 coincided with the region of qTDW1.2 for total seedling dry weight and qRDW1.1 for root dry weight [8]. The qSDW2 was on the similar location of qGP-2 for germination percentage [12], and the qSDW3 was similar with the location of qIR-3 for imbibition rate [12] and q7GR for germination rate [24]. The qSDW5 mapped near the region of qTDW5-2 for total dry weight and qTAA5-1 for total amylase activity [8], and the qSDW8.3 was on the similar location of qIR-8 for imbibition rate [12]. The qSDW9 identified here was near the region of qRA9-1 for root activity and qSW9-1 for seed weight [8], and the qSDW12 and qWMSR12 was near the region of qSH12.1 for shoot height [20] and qIR-12 for imbibition rate [12].

Similarly, the qWMSR2 and qSRUE2.2 was on the similar location of qGR-2 for germination rate [11], gw2.1 for 1000-seed weight [51] and qGW2d for grain width [52]. The qWMSR3 located to a position that coincided with the region of qLTG-3 for low temperature germinability [25]. The qWMSR7.1 was on the similar location of qGR7-1 [8] and q7GR [24] for germination rate,
qGI-7 for germination index [11] and qGP-7.1 for germination percentage [12]. The qWMSR10 mapped near the region of qIR-10 for imbibition rate [12].

Additionally, the qSRUE1 identified here was near the region of qTDW1-1 for total dry weight, qSDW1-1 for shoot dry weight, qSW1-1 for seed weight [8] and qSH1.3 for shoot height [20]. The qSRUE3 was near the region of qLTG-3-2 for low temperature germinability [22]. The qSRUE4.1 was on the similar location of qGP-4.1 for germination rate [12] and qLTG-4 for low temperature germinability [22], and the qSRUE4.2 was similar with the region of qSH4 for shoot height [20]. The qSRUE4.4 located on the same region of qSD4 for seed dormancy [53], qLTG-4 for low temperature germinability [25] and qGP-4 for germination percentage [11]. The qSRUE4.5 was on the similar location of qGP-4.2 for germination percentage and qIR-4 for imbibition rate [13]. The qSRUE12.2 mapped near the region of qDSW12.1 for seedling dry weight [12]. However, there were no QTLs previously reported to be close to qSDW4.1, qSDW4.2, qSDW8.1, qSDW8.2, qWMSR4, qWMSR7.2, qSRUE4.1, qSRUE4.3, qSRUE8.1, qSRUE8.2, and qSRUE12.1, which indicates that these additive QTLs might be novel (Fig. 4).

Molecular evidence indicates that genes related to reserve mobilization and endosperm weakening could possibly affect seed germination [54–55]. Recently, several major QTLs for seed germination have been identified. For example, Wang et al. [11] identified one major QTL (qGI-11) for seed germination speed. Wang et al. [12] identified one major QTL (qGR-2) for seed germination under salt stress conditions. Recently, examination of the mechanism of seed vigor in rice has made significant progress. Fujino et al. [22] mapped a major rice QTL (qLTG3-1) for low-temperature germinability on chromosome 3. Fujino et al. [23] placed qLTG3-1 in a 4.8-kb region of chromosome 3 using high-resolution mapping and revealed that the expression of qLTG3-1 was tightly associated with the vacuolation of the tissues covering Figure 4. The co-located QTLs in this study with previously mapped QTLs in rice. The mapped QTLs in this study are shown on the left (red) and previously mapped QTLs are shown on the right (green) [8,11–13,19–25,51–53]. doi:10.1371/journal.pone.0080002.g004

Figure 4. The co-located QTLs in this study with previously mapped QTLs in rice.
the embryo. This vacuolation may cause tissue weakening, resulting in the reduction of the mechanical resistance to the growth potential of the coleoptile. Seed reserve utilization is a very complex physiological process during seed germination. In this study, two major QTLs qWMSR1.7 and qWUE1.3 were identified, and each QTL could explain more than 20% of the total phenotypic variance. Fine mapping the two QTLs are now in progress to elucidate the molecular mechanism of seed reserve utilization using near isogenic lines (NILs). Furthermore, the linked marker with the two major QTLs might be useful in marker-assisted selection to increase the seed reserve utilization in rice.

In a hypothetical cross of two cultivars, the trait values of produced RILs can be predicted by the effects of all the detected loci. The best RIL with maximum value would represent the best cross [41]. In this study, the four best cross combinations of each trait for the development of RIL populations were selected. It was found that some RILs present repeatedly in the novel parental combinations for example, RIL 1750 in SDW and WMSR and RIL 1890 in WMSR and SREU, indicating high general combining ability. To improve multiple traits, all the elite alleles might be pyramided into one cultivar as far as possible [41]. In this study, a total of 33 elite alleles could be pyramided by the combination of four RILs to improve the seed reserve utilization traits. These results showed that the selected RILs as new materials will be valuable in future rice breeding programs. The identified QTLs will be applicable to improve rice seed reserve utilization by MAS.

Author Contributions
Conceived and designed the experiments: ZW HZ. Performed the experiments: XC JC. Analyzed the data: JC XH YL. Contributed reagents/materials/analysis tools: LW WD. Wrote the paper: ZW.

References
1. Ichia T, Nonomiya I, Ogino K (2000) Utilization of seed reserves during germination and early seedling growth by Ditylenchus dipsaci (Dipetalonematidae). J Trop Ecol 17: 371–378.
2. Finch-Savage WE, Clay HA, Lynn JR, Morris K (2010) Towards a genetic understanding of seed vigour in small-seeded crops using natural variation in Phaseolus vulgaris. Plant Physiol 153: 1582–1590.
3. Hodgkin T, Hegarty TW (1978) Genetically determined variation in seed germination and field emergence of Brachyscome iberidifolia. Ann Appl Biol 88: 407–415.
4. Guan YJ, Hu J, Wang XJ, Zhao CX (2009) Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. J Zhejiang Univ Sci B 10: 427–433.
5. Schwemmer AR, Bradford KJ (2010) A genetic locus and gene expression patterns associated with the priming effect on lettuce seed germination at elevated temperatures. Plant Mol Biol 73: 117–131.
6. Chen K, Arora R (2011) Dynamics of the antioxidant system during seed osmopriming, post-priming germination, and seedling establishment in Spinach (Spinacia oleracea). Plant Sci 180: 212–220.
7. Zhang M, Wang Z, Yuan L, Yin C, Cheng J, et al. (2012) Osmopriming improves tomato seed vigor under aging and salinity stress. Afr J Biotechnol 11: 6305–6311.
8. Cui K, Peng S, Xing Y, Xu C, Yu S, et al. (2002) Molecular dissection of seedling-vigor and associated physiological traits in rice. Theor Appl Genet 105: 745–753.
9. Foulds MR, Zhang LP, Subbiah P (2003) Genetics of drought tolerance during seed germination in tomato: inheritance and QTL mapping. Genome 46: 536–545.
10. Hayashi E, Aoyama N, Still DW (2008) Quantitative trait loci associated with aging on subsequent seed reserve utilization and seedling growth in soybean. J Trop Ecol 17: 371–378.
11. Wang Z, Wang J, Yang F, Wang H, Zhang HS (2010) Quantitative trait locus analysis for rice seed vigor during the germination stage. J Zhejiang Univ Sci B 11: 956–964.
12. Wang Z, Wang J, Bao Y, Wu Y, Zhang H (2011) Quantitative trait loci controlling rice seedling growth under salinity stress. Euphytica 178: 297–307.
13. Wang Z, Wang J, Zhou R, Wang J, Zhang H (2011) Identification of quantitative trait loci for cold tolerance during the germination and seedling stages in rice (Oryza sativa L.). Euphytica 181: 405–413.
14. Alonso-Blanco C, Aarts MGM, Bennink J, Keurentjes JB, Reymond M, et al. (2005) What has natural variation taught us about plant development, physiology and adaptation. Plant Cell 21: 1877–1896.
15. Pritchard SL, Charleston WL, Baker A, Graham IA (2002) Germination and storage reserve mobilization are regulated independently in Arabidopsis. Plant J 31: 639–647.
16. Solani A, Gholipoor M, Zeinali E (2006) Seed reserve utilization and wheat as affected by drought and salinity. Environ Exp Bot 55: 195–200.
17. Mohammadi H, Solani A, Sadeghroro HH, Zeinali E (2011) Effects of seed aging on subsequent seed reserve utilization and seedling growth in soybean. Int J Plant Prod 5: 65–70.
18. Solani A, Galeshi S, Zeinali E, Lusti N (2002) Germination, seed reserve utilization and seedling growth of chickpea as affected by salinity and seed size. Seed Sci Technol 30: 53–60.
19. Wang Z, Chen Z, Cheng J, Lai Y, Wang J, et al. (2012) QTL analysis of Na+ and K+ concentrations in roots and shoots under different levels of NaCl stress in rice (Oryza sativa L.). PLoS ONE 7: e51202.
20. Wang Z, Cheng J, Chen Z, Huang J, Bao Y, et al. (2012) Identification of QTLs with main, epistatic and QTL × environment interaction effects for salt tolerance in rice seedlings under different salinity conditions. Theor Appl Genet 125: 807–815.
is independent of abscisic acid, and requires PHOSPHOENOLPYRUVATE CARBOXYKINASE1. Plant Cell 16: 2705–2718.

43. Aoki N, Scofield GN, Wang XD, Offler CE, Patrick JW, et al. (2006) Pathway of sugar transport in germinating wheat seeds. Plant Physiol 141: 1255–1263.

44. Leonova S, Grünberg A, Martula S, Stymne S, Carlsson AS (2010) Mobilization of lipid reserves during germination of oat (Avena sativa L.), a cereal rich in endosperm oil. J Exp Bot 61: 3089–3099.

45. Soltani A, Zeimali E, Galeshi S, Latifi N (2001) Genetic variation for and interrelationships among seed vigor traits in wheat from the Caspian Sea coast of Iran. Seed Sci Technol 29: 653–662.

46. Douglas CL, Wilkins DE, Churchill DB (1994) Tillage, seed size and seed density effects on performance of soft white winter wheat. Agron J 86: 707–711.

47. Bockous WW, Shroyer JP (1996) Effect of seed size on seedling vigor and forage production of winter wheat. Can J Plant Sci 76: 101–105.

48. Carleton AE, Cooper CS (1972) Seed size effects upon seedling vigor of three forage legumes. Crop Sci 12: 183–186.

49. Johnson DR, Wax LM (1976) Relationships of soybean germination and vigor tests to field performance. Agron J 70: 273–278.

50. Shroyer JP, Cox TS (1984) Effects of cultivar, environment and their interaction on seed quality of hard red winter wheat from production fields. J Appl Seed Prod 2: 24–28.

51. Marri PR, Sarla N, Reddy LV, Siddiq EA (2005) Identification and mapping of yield and yield related QTLs from an Indian accession of Oryza sativa. BMC Genet 6: 33.

52. Bai X, Luo L, Yan W, Kovi MR, Zhan W, et al. (2010) Genetic dissection of rice grain shape using a recombinant inbred line population derived from two contrasting parents and fine mapping a pleiotropic quantitative trait locus qGd7. BMC Genet 11: 16.

53. Gu XY, Kianian SF, Foley ME (2004) Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (Oryza sativa). Genetics 166: 1503–1516.

54. Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, et al. (2006) Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. Plant Physiol 142: 839–854.

55. Bethke PC, Liboureil RG, Aoyama N, Chung YY, Still DW, et al. (2007) The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiol 143: 1173–1188.