A Fast Multi-Locus Ridge Regression Algorithm for High-Dimensional Genome-Wide Association Studies

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The mixed linear model (MLM) has been widely used in genome-wide association study (GWAS) to dissect quantitative traits in human, animal, and plant genetics. Most methodologies consider all single nucleotide polymorphism (SNP) effects as random effects under the MLM framework, which fail to detect the joint minor effect of multiple genetic markers on a trait. Therefore, polygenes with minor effects remain largely unexplored in today's big data era. In this study, we developed a new algorithm under the MLM framework, which is called the fast multi-locus ridge regression (FastRR) algorithm. The FastRR algorithm first whitens the covariance matrix of the polygenic matrix K and environmental noise, then selects potentially related SNPs among large scale markers, which have a high correlation with the target trait, and finally analyzes the subset variables using a multi-locus deshrinking ridge regression for true quantitative trait nucleotide (QTN) detection. Results from the analyses of both simulated and real data show that the FastRR algorithm is more powerful for both large and small QTN detection, more accurate in QTN effect estimation, and has more stable results under various polygenic backgrounds. Moreover, compared with existing methods, the FastRR algorithm has the advantage of high computing speed. In conclusion, the FastRR algorithm provides an alternative algorithm for multi-locus GWAS in high dimensional genomic datasets.

Keywords: genome-wide association study, mixed linear model, multi-locus algorithm, statistical power, polygenic background, minor effect

INTRODUCTION

Genome-wide association study (GWAS) has been widely used in the genetic dissection of quantitative traits in human, animal, and plant genetics. GWAS typically searches for the correlations between genetic variants and hundreds or thousands of individuals. However, a complete characterization of the biological mechanism for most quantitative traits remains elusive.

Abbreviations: MLM, the mixed linear model; GWAS, genome-wide association study; FastRR, fast multi-locus ridge regression; SNP, single nucleotide polymorphism; QTN, quantitative trait nucleotide; EMMA, efficient mixed model association; DRR, deshrinking ridge regression; ORR, ordinary ridge regression; BLUP, best linear unbiased prediction; lasso, least absolute shrinkage and selection operator; SCAD, smoothly clipped absolute deviation; DEMMA, Decontaminated efficient mixed model association; MAF, minor allele frequency; LD, days to flowering under long days; SD, days to flowering under short days; SDV, days to flowering under short days with vernalization; MSE, mean squared error.
provide the tools for high-dimensional genetic data analysis. It is known that the quantitative traits are controlled by a few genes with large effects and numerous polygenes with minor effects. Nevertheless, the dissection of the polygenes with minor effects needs to be improved in above mentioned multi-locus approaches.

In this study, we propose a multi-stage flexible approach for GWAS to detect the associated (large and minor effects) variables/SNPs. In our model, the fast multi-locus ridge regression algorithm (FastRR), all SNP effects are considered as random effects. The FastRR algorithm first whitens the covariance matrix of the polygenic matrix K and environmental noise. Subsequently, the FastRR algorithm reduces the number of SNPs according to correlation, the variables of which significantly correlate with the response are retained for the next stage. In the final stage, deshrinking ridge regression (DRR) is applied to implement parametric estimation and significance tests of variables. In this study, a series of simulated and real dataset analyses are used to validate this new method. For comparison, five established methods – lasso, adaptive lasso, smoothly clipped absolute deviation (SCAD), EMMA, and decontaminated efficient mixed model association (DEMM) are used for analysis.

**MATERIALS AND METHODS**

**Genetic Model**

Let $y_{i}$ (i = 1, 2, ..., n) be the phenotypic value of the i-th individual in a sample of size n from a natural population, and the genetic model can be described as:

$$y = W\alpha + Z\gamma + u + \varepsilon$$

where $y = (y_1, y_2, ..., y_n)^T$; $\alpha$ is a $c \times 1$ vector of the fixed effects, such as the intercept, population structure effect and so on, W is the corresponding designed matrix for $\alpha$; $Z$ is an $n \times 1$ vector of marker genotypes, and $\gamma \sim N(0, \sigma_\gamma^2)$ is a random effect of putative QTN. $\sigma_\gamma^2$ is the variance of the putative QTN; $u \sim MVN(0, \sigma_u^2 \mathbf{K})$ is an $n \times 1$ random vector of polygenic effects, $\sigma_u^2$ is the variance of polygenic background, $\mathbf{K}$ is a known $n \times n$ relatedness matrix; $\varepsilon$ is an $n \times 1$ vector of residual errors with an assumed MVN$(0, \sigma_\varepsilon^2 \mathbf{I}_n)$ distribution; $\sigma_\varepsilon^2$ is the variance of residual error; and $\mathbf{I}_n$ is a $n \times n$ identity matrix. $\text{MVN}$ denotes multivariate normal distribution.

As $\gamma$ is treated as being a random effect, the variance of $y$ in the model (1) is:

$$\text{var}(y) = \sigma_\gamma^2 Z Z^T + \sigma_u^2 \mathbf{K} + \sigma_\varepsilon^2 \mathbf{I}_n = \sigma^2(\lambda_\gamma Z Z^T + \lambda_u \mathbf{K} + \mathbf{I}_n)$$

where $\lambda_\gamma = \sigma_\gamma^2 / \sigma^2$, $\lambda_u = \sigma_u^2 / \sigma^2$.

**Fast Multi-Locus Ridge Regression Algorithm**

The FastRR algorithm is a multi-stage flexible approach for GWAS, which simultaneously implements estimation and testing
to detect associated variables/SNPs. We describe it with the following stages:

**The Polygenic and Residual Noise Whitening Stage**

The key point of solving the model (1) is to estimate two ratios of variance components, $\lambda_\gamma$ and $\lambda_g$, which cause expensive computational burden. It is noted that polygenic variance is always larger than zero, while variance components for most SNPs are zero because these markers are not associated with the interested trait, which is $\lambda_g = 0$ for most SNPs. Therefore, in the first step, we estimate $\lambda_g$ by the reduced form of the model (1), which deleted $Z_\gamma$ with only polygenic background, and replace $\lambda_g$ in (2) by the $\hat{\lambda}_g$ (Wen et al., 2018, 2020), avoiding re-estimate $\lambda_g$ for each single marker scanning. Thus,

$$\text{var}(y) = \sigma^2(\lambda_\gamma ZZ^T + \hat{\lambda}_g K + I_n) = \sigma^2(\lambda_\gamma ZZ^T + B) \quad (3)$$

An eigen (or spectral) decomposition of the positive definite matrix $B = \hat{\lambda}_g K + I_n$ is:

$$B = QA\Lambda Q^T = (QA^{\frac{1}{2}}Q^T)(QA^{\frac{1}{2}}Q^T) \quad (4)$$

where $Q$ is orthogonal and $\Lambda$ is a diagonal matrix with positive eigenvalues. Let $C = QA^{-\frac{1}{2}}Q^T$, the model (1) is changed to:

$$y_c = W_c \alpha + Z_c \gamma + \epsilon_c \quad (5)$$

where, $y_c = Cy$, $W_c = CW$, $Z_c = CZ$, $\epsilon_c = Cu + Ce \sim \text{MVN}(0, \sigma^2 I_n)$ (Wen et al., 2018, 2020).

**Variable Reduction Stage**

A number of studies have illustrated that most quantitative traits are controlled by a small portion of genes, including a few genes with large effects and polygenes with minor effects (Zhang et al., 2017; Wen et al., 2019). It is critical to dissect all associated loci from large-scale genetic markers. Herein, we conduct a variable reduction stage, whose purpose is dimension reduction. At this stage, the FastRR algorithm detects a subset of putative variables associated with the phenotype, and thus avoids the intractable computational problems of high-dimensional datasets analysis.

We calculate the marginal correlation coefficients between $Z_c$ (variables after polygenic background correction) and $y_c$ (phenotype after polygenic background correction) under model (5), R function cor.test returns the p-value of the correlation test. The critical value for significance was set at p-value < 0.01 (Tamba et al., 2017). For the threshold of 0.01, even the slight correlations between predictors and the response will be captured (Tamba et al., 2017), and the unassociated loci will be removed. All the most potential QTNs are selected to construct the reduced multi-locus model for the next stage. Essentially, this marginal correlation step is similar to the single marker scanning, which combined with the polygenic background without considering variance components $\sigma^2_\gamma$.

**Parameter Estimation Stage**

In the multi-locus model,

$$y = W\alpha + Z\gamma + \epsilon \quad (6)$$

where $y$ is the phenotypic value of the quantitative trait, which is the same as that in the model (1); $\alpha$ is a vector of fixed effects, $y$ is a $q \times 1$ random effect vector of the selected $q$ markers from the above stage, and $\gamma_k \sim N(0, \phi^2)$ $k = 1, ..., q$; $W$ and $Z$ are the corresponding design matrices for $\alpha$ and $\gamma$. Here, polygenic background correction is not considered in model (6), because the above two steps under the polygenic background model had already selected all potential associated QTNs. All the parameters in model (6) are estimated by DRR proposed by Wang et al. (2020).

Before introducing the DRR, let us briefly recall the ordinary ridge regression (ORR). According to the best linear unbiased prediction (BLUP) of the marker effects and the prediction error variances using the conditional expectation and conditional variance, the estimates of ORR are as follows,

$$\hat{\gamma}_{ORR} = E(\gamma | y) = \lambda Z^T H^{-1}(y - W\alpha) \quad (7)$$

$$\text{var}(\hat{\gamma}_{ORR}) = \left(\lambda I - \lambda Z^T H^{-1}Z\right) \quad (8)$$

where $\lambda = \frac{\phi^2}{\sigma^2}$, $H = (ZZ^T)^{\frac{1}{2}} \lambda + I_n$.

Ordinary ridge regression is inflexible and inaccurate for GWAS (Wang et al., 2020). Therefore, we apply the following DRR method, which can bring both the accurate effects and tests back. The essential difference between ORR and DRR is the well-measurement-factor (also called degree of freedom), which is $d_k = 1 - \frac{\text{var}(\hat{\gamma}_{ORR})}{\phi^2} = \lambda Z_k^T H^{-1}Z_k \quad (9)$

$\hat{\gamma}_{ORR}$ is the $k$-th element of $\hat{\gamma}_{ORR}$, where $\phi^2$ and $\text{var}(\hat{\gamma}_{ORR})$ are prior and posterior variances for $\gamma_k$, respectively.

$$\hat{\gamma}_{k}^{DRR} = \frac{\phi^2}{\phi^2 - \text{var}(\hat{\gamma}_{k}^{ORR})} \hat{\gamma}_{ORR} = d_k^{-1} \hat{\gamma}_{ORR} \quad (10)$$

$$\text{var}(\hat{\gamma}_{k}^{DRR}) = \frac{\phi^2}{\phi^2 - \text{var}(\hat{\gamma}_{k}^{ORR})} \text{var}(\hat{\gamma}_{k}^{ORR}) \quad (11)$$

$$W_k = \frac{(\hat{\gamma}_{k}^{DRR})^2}{\text{var}(\hat{\gamma}_{k}^{DRR})} = \frac{(\hat{\gamma}_{k}^{ORR})^2}{\text{var}(\hat{\gamma}_{k}^{ORR}) / d_k^2} = d_k^{-1} \frac{(\hat{\gamma}_{k}^{ORR})^2}{\text{var}(\hat{\gamma}_{k}^{ORR})} \quad (12)$$

The test statistic of DRR, $W_k$, follows a Chi-square distribution with one degree of freedom under the null model, $H_0 : \gamma_k = 0$. The DRR method deshrinks both the estimated effects of markers and their estimated variances from the ORR, resulting in deshrunk Wald test statistics.

**Comparison Methods**

**LASSO**

Lasso regression (Tibshirani, 1996) is a type of linear regression that implements shrinkage by performing $L_1$ regularization and
selects the most correlated with response variables. It is a popular method for simultaneous estimation and variable selection. The method was implemented by the R software package `lars`.

**Adaptive Lasso**

Similar to the lasso, the adaptive lasso (Zou, 2006) is a mainstream method of variable selection, in which the adaptive weights are used for penalizing different coefficients in the $L_1$ penalty. Adaptive lasso shows more consistence for variable selection than lasso in data analysis. The method was implemented by the R software package `glmnet`.

**SCAD**

SCAD (Fan and Li, 2001) as the variable selection has the nice oracle property. The estimator of SCAD attempts to alleviate bias from variable selection, while also retaining a continuous penalty that encourages sparsity. The method was implemented by the R software package `ncvreg`.

**EMMA**

Efficient mixed-model association (Kang et al., 2008) is an established genome-wide single-marker scan methodology under the framework of MLM, in which the polygenic background and population structure are controlled. The method was implemented by the R software package EMMA.

**DEMMA**

The polygenic effect (the sum of all marker effects) is treated as a random effect in EMMA. On the other side, EMMA already included the marker effect as the fixed effect. Thus, there are two effects for each marker, which lead to a reduced power for testing. Wang et al. (2020) proposed DEMMA to overcome the above drawback. The method was implemented by the R code.

### Experimental Materials

#### The Simulation Data

Three Monte Carlo simulation experiments were conducted to evaluate the performances of the FastRR algorithm and other methods. We generated genotypes according to the minor allele frequency (MAF) in the interval $(0.1, 0.5)$ under Hardy–Weinberg equilibrium. The simulation datasets contained $n = 2000$ individuals with $p = 10,000$ genetic variants, which were generated with MLM. The total average was set at 10.0 and residual variance was set at 10.0. We considered three scenarios for each simulation, including two times polygenic background, five times polygenic background, and ten times polygenic background.

Only one QTN with a fixed position (Table 1) was simulated and placed on the SNPs with 0.1 heritability for the first simulation; five QTNs with fixed positions were assigned and placed on the SNPs for the second simulation, the heritabilities of the QTNs were set as 0.02, 0.05, 0.05, 0.08, and 0.10, respectively. The numbers in parentheses represent the standard deviation.

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1. https://cran.r-project.org/web/packages/lars/index.html
2. https://glmnet.stanford.edu/
3. https://cran.r-project.org/web/packages/ncvreg/index.html
4. http://mouse.cs.ucla.edu/emma/
5. https://doi.org/10.1093/bioinformatics/btaa345/
### TABLE 2A | Comparison of lasso, adaptive lasso, SCAD, EMMA, DEMMA, and FastRR methods in the second simulation experiment (scenarios 1: two times polygenic background).

| QTN | True value | Lasso | Adaptive lasso | SCAD | EMMA | DEMMA | FastRR |
|-----|------------|-------|----------------|------|------|-------|--------|
|     |            |       |                |      |      |       |        |
|     | Position   | Effect | r²             | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE |
| Polygenic background (2K) |
| 1   | 98         | 0.5451 | 2%             | 99.0 (0.091) | 6.833 | 96.0 (0.149) | 3.703 | 99.0 (0.122) | 9.011 | 91.0 (0.087) | 0.269 | 94.0 (0.089) | 0.956 | 99.0 (0.084) | 0.596 | 0.978 |
| 2   | 301        | 0.8622 | 5%             | 100.0 (0.100) | 9.080 | 100.0 (0.114) | 1.924 | 100.0 (0.174) | 6.221 | 100.0 (0.095) | 0.822 | 100.0 (0.095) | 1.044 | 100.0 (0.094) | 0.820 | 1.054 |
| 3   | 540        | 0.8598 | 5%             | 100.0 (0.093) | 7.350 | 100.0 (0.101) | 1.240 | 100.0 (0.150) | 3.906 | 100.0 (0.089) | 0.852 | 100.0 (0.089) | 0.788 | 100.0 (0.089) | 0.850 | 0.788 |
| 4   | 801        | 1.0789 | 8%             | 100.0 (0.099) | 8.34 | 100.0 (0.105) | 1.333 | 100.0 (0.139) | 2.211 | 100.0 (0.094) | 1.061 | 100.0 (0.094) | 0.914 | 100.0 (0.094) | 1.059 | 0.911 |
| 5   | 1000       | 1.2093 | 10%            | 100.0 (0.096) | 7.276 | 100.0 (0.098) | 1.023 | 100.0 (0.251) | 0.886 | 100.0 (0.094) | 1.223 | 100.0 (0.094) | 0.886 | 100.0 (0.094) | 1.220 | 0.878 |

False positive rate (%) 0.461 0.024 0.355 0.000 0.007 0.422

Three scenarios, including two times polygenic background, five times polygenic background, and ten times polygenic background.

MSE, mean squared error.
The numbers in parentheses represent the standard deviation.

### TABLE 2B | Comparison of lasso, adaptive lasso, SCAD, EMMA, DEMMA, and FastRR methods in the second simulation experiment (scenarios 2: five times polygenic background).

| QTN | True value | Lasso | Adaptive lasso | SCAD | EMMA | DEMMA | FastRR |
|-----|------------|-------|----------------|------|------|-------|--------|
|     |            |       |                |      |      |       |        |
|     | Position   | Effect | r²             | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE | Power (%) | Effect (SD) | MSE |
| Polygenic background (5K) |
| 1   | 98         | 0.5451 | 2%             | 89.0 (0.091) | 9.048 | 86.0 (0.166) | 3.703 | 99.0 (0.149) | 9.011 | 91.0 (0.087) | 0.269 | 94.0 (0.089) | 0.956 | 99.0 (0.084) | 0.596 | 0.978 |
| 2   | 301        | 0.8622 | 5%             | 100.0 (0.119) | 12.673 | 100.0 (0.166) | 3.703 | 100.0 (0.200) | 10.515 | 99.0 (0.106) | 0.841 | 100.0 (0.106) | 1.140 | 100.0 (0.106) | 0.820 | 1.283 |
| 3   | 540        | 0.8598 | 5%             | 100.0 (0.117) | 13.063 | 100.0 (0.153) | 3.439 | 100.0 (0.191) | 10.812 | 99.0 (0.107) | 0.831 | 100.0 (0.110) | 1.195 | 100.0 (0.110) | 0.828 | 1.299 |
| 4   | 801        | 1.0789 | 8%             | 100.0 (0.116) | 11.824 | 100.0 (0.126) | 1.811 | 100.0 (0.186) | 4.911 | 100.0 (0.117) | 1.077 | 100.0 (0.117) | 1.336 | 100.0 (0.116) | 1.075 | 1.334 |
| 5   | 1000       | 1.2093 | 10%            | 100.0 (0.109) | 10.937 | 100.0 (0.117) | 1.480 | 100.0 (0.150) | 2.428 | 100.0 (0.101) | 1.234 | 100.0 (0.101) | 1.063 | 100.0 (0.100) | 1.232 | 1.049 |

False positive rate (%) 0.461 0.024 0.355 0.000 0.007 0.422

Three scenarios, including two times polygenic background, five times polygenic background, and ten times polygenic background.

MSE, mean squared error.
The numbers in parentheses represent the standard deviation.
### Table 2C

Comparison of lasso, adaptive lasso, SCAD, EMMA, DEMMA, and FastRR methods in the second simulation experiment (scenarios 3: ten times polygenic background).

| QTN  | True value | Lasso  | Adaptive lasso | SCAD  | EMMA | DEMMA | FastRR |
|------|------------|--------|----------------|-------|-------|-------|--------|
|      | Power (SD) | Power (SD) | Power (SD) | Power (SD) | Power (SD) | Power (SD) | Power (SD) |
|      | Effect (MSE) | Effect (MSE) | Effect (MSE) | Effect (MSE) | Effect (MSE) | Effect (MSE) | Effect (MSE) |
| Polygenic background (10K) | | | | | | |
| 1    | 98.0 | 0.0451 | 2% | 96.0 | 0.223 | 51.0 | 0.346 | 10.0 | 0.867 |
|      | 4.297 | 51.0 | 0.240 | 5.165 | 20.0 | 0.757 | 1.98 | 0.5451 | 2% |
| 2    | 301.0 | 0.8022 | 5% | 97.0 | 0.459 | 17.500 | 97.0 | 0.726 | 12.40 |
|      | 3.287 | 100.0 | 0.855 | 11.254 | 100.0 | 1.020 | 0.862 | 0.209 |
| 3    | 540.0 | 0.6858 | 5% | 97.0 | 0.459 | 17.500 | 97.0 | 0.726 | 12.40 |
|      | 3.592 | 100.0 | 0.855 | 11.254 | 100.0 | 1.020 | 0.862 | 0.209 |
| 4    | 801.0 | 1.0769 | 8% | 100.0 | 0.862 | 17.912 | 97.0 | 0.726 | 12.40 |
|      | 1.2083 | 100.0 | 1.020 | 0.129 | 1.129 | 0.1173 | 0.1094 | 0.059 |
| 5    | 1000.0 | 1.2083 | 10% | 100.0 | 0.783 | 20.627 | 96.0 | 1.129 | 0.1173 |

### Results

The Rice Data

To validate the FastRR algorithm, the rice data that was used in this study for GWAS demonstration consists of 524 inbred varieties, which were collected from China and southeast Asia (Chen et al., 2014; Wei et al., 2018). A total of 6.5 million high-quality SNPs covering 90% of total SNPs were analyzed by Chen et al. (2014). A total of 314,393 SNPs and grain width traits (Wang et al., 2020) were analyzed in this study. These data were downloaded from the link.4

The Arabidopsis Data

To further evaluate the performance of FastRR, we reanalyzed the genetic data sets of Arabidopsis published by Atwell et al. (2010). Both phenotypes and genotypes were obtained from the link.5 A total of 199 Arabidopsis lines and 216,130 SNPs were used for analysis. Among all traits, we analyzed three traits related to flowering time: (1) LD: days to flowering under long days; (2) SD: days to flowering under short days; and (3) SDV: days to flowering under short days with vernalization.8

### Statistical Power for QTN Detection

#### Simulation Studies

To further evaluate the performance of FastRR, we reanalyzed the genetic data sets of Arabidopsis published by Atwell et al. (2010). Both phenotypes and genotypes were obtained from the link. A total of 199 Arabidopsis lines and 216,130 SNPs were used for analysis. Among all traits, we analyzed three traits related to flowering time: (1) LD: days to flowering under long days; (2) SD: days to flowering under short days; and (3) SDV: days to flowering under short days with vernalization.8

8https://doi.org/10.1093/bioinformatics/btaa345/

9http://www.arabidopsis.usc.edu/
The results illustrate that the trends are similar to the above experiments (Figure 3). In summary, the FastRR algorithm retains an obviously advantageous performance for the random loci experiment. These results demonstrate the highest power of the FastRR algorithm across all the approaches under various genetic backgrounds.

Accuracy for the Estimated QTN Effects

The average effect and mean squared error (MSE) are used to measure the accuracy of an estimated QTN effect. We evaluated the accuracies for the (fixed positions, including simulation experiment 1 and 2) estimates using all six methods (Tables 1, 2A–C). As a result, the estimates for each QTN effect for EMMA, DEMMA, and FastRR are much closer to the true value, and EMMA and DEMMA are slightly better than the FastRR algorithm, nevertheless, EMMA and DEMMA methods have relatively lower power than FastRR. The performance of SCAD, adaptive lasso, and lasso are unsatisfactory. The MSE shows a similar trend to the average effect. On these occasions, the FastRR algorithm, EMMA, and DEMMA methods are recommended for the estimation of QTN effects.

The false positive rate is a crucial index in GWAS. All the false positive rate results of simulation experiment 1 and 2 are listed in Tables 1, 2A–C. Obviously, the false positive rate becomes increasingly high along with the stronger polygenic background. EMMA, DEMMA, and adaptive lasso have a relatively lower false positive rate followed by FastRR, SCAD, and lasso. The false positive rates of all six methods are under control.

Computing Time

We compare the computing time of 100 repeated simulated analyses by using six approaches. In each of the three simulation experiments, computing times are recorded and are shown in Figure 4 and Supplementary Figures 1, 2 (Intel Xeon E5-2630...
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FIGURE 3 | The average statistical powers for all QTNs in the third simulation experiment using six methods (lasso, adaptive lasso, SCAD, EMMA, DEMMA, and the FastRR algorithm).

FIGURE 4 | Comparison of computing times to analyze simulation experiment 1 using all six methods (lasso, adaptive lasso, SCAD, EMMA, DEMMA, and the FastRR algorithm).

The average computing time in seconds for the FastRR algorithm is significantly lower than for the other methods. The FastRR algorithm is more efficient in terms of computing time.

Analysis of the Rice Data Set

To validate the FastRR algorithm, the grain width trait of rice data is analyzed by using six methods: lasso, adaptive lasso, SCAD, EMMA, DEMMA, and the FastRR algorithm. The rice dataset contains 310,000 SNPs genotyped for 524 inbred varieties. Supplementary Figure 3 shows the LOD plot for three variable selection methods and Manhattan plots for the other three methods. Obviously, DEMMA method and the FastRR algorithm have the identical detected regions, two significant peaks on chromosome 5 and 9. Both DEMMA and FastRR detect the cloned gene GW5 (Weng et al., 2008) that controls grain width trait. The test statistics of SNP135176 (the most significant SNP) for the DEMMA method and FastRR algorithm are $2.31 \times 10^{-26}$ and $1.92 \times 10^{-20}$, respectively; the $p$-value for the DEMMA method is lower than for the FastRR algorithm. However, the test statistics for the EMMA method do not reach the Bonferroni correction threshold. In addition, three variable selection methods, lasso, adaptive lasso, and SCAD, show unsatisfactory performance according to the LOD scores.

The average computing times are listed in Table 3. The relatively fast methods, lasso, SCAD, and FastRR, are 235.33, 455.31, and 561.31 s, respectively. Lasso is the fastest method.
TABLE 3 | The computation times (seconds) for analyzing Arabidopsis flowering time traits and rice grain width by using lasso, adaptive lasso, SCAD, EMMA, DEMMA, and FastRR methods.

| Traits         | Lasso | Adaptive lasso | SCAD   | EMMA         | DEMMA | FastRR |
|----------------|-------|----------------|--------|--------------|-------|--------|
| Rice Grain width | 235.33 | 1067.22 | 455.31 | 60813.82     | 26417.71 | 561.31 |
| Arabidopsis LD   | 36.11  | 189.36 | 128.79 | 1362.55      | 1117.49 | 105.17 |
| SD              | 37.17  | 159.00 | 114.17 | 1350.19      | 4114.88 | 112.75 |
| SDV             | 44.47  | 140.96 | 112.34 | 1665.94      | 4123.34 | 107.36 |

among all six methods, which is followed by SCAD and FastRR. In Table 3, the adaptive lasso is different from SCAD and FastRR. The computation times for analyzing Arabidopsis flowering time traits and rice grain width by using lasso, adaptive lasso, SCAD, EMMA, DEMMA, and FastRR methods.

Analysis of the Arabidopsis Data Set

To further validate the FastRR algorithm, this new algorithm FastRR along with lasso, adaptive lasso, SCAD, EMMA, and DEMMA methods are used to reanalyze the Arabidopsis data for three traits related to flowering time (LD, SD, and SDV). The results are illustrated in Supplementary Figures 4–6. Each putative QTN (over the threshold) is used to mine the candidate genes by The Arabidopsis Information Resource®. The FastRR algorithm detects the confirmed genes AGL17 and CDKG1, which are detected by SCAD and DEMMA as well. From the analysis results, lasso shows several false positive loci in the detection of SD and SDV, meanwhile the adaptive lasso and SCAD methods are inflexible in dissecting the SNPs associated with the target traits. The statistical tests of EMMA are under the Bonferroni corrected threshold. The FastRR algorithm shows a similar pattern as the DEMMA method for all results of three traits, the statistics of part SNPs using the DEMMA method are slightly more significant than the FastRR algorithm, which is similar to the results of the rice datasets.

In terms of the computing speed for all three traits, lasso is computationally much faster than the other methods. The computing times of FastRR, SCAD, and adaptive lasso are on the same order of magnitude, which require less than 200 s. The DEMMA and EMMA methods have much more computational burden than the other methods, both of which require over ten times the computing time required by the FastRR algorithm. Overall, the FastRR algorithm is recommended from the perspective of detection and computing speed across all experiments.

DISCUSSION

The FastRR algorithm is a multi-stage flexible approach for QTNS dissection in GWAS, and displays high power for detecting QTN of large and minor effects, even under the ten times polygenic background. We aimed to understand the performance of regression analysis methods, thus the following three regression analysis methods, ORR, DRR, and FastRR, are used to analyze simulation experiment 1 and 2. As the results show (Supplementary Tables 1, 2A–C), ORR has the worst detection ability, and even major QTN with large effects are not identified. This explains why ORR is rarely used in GWAS. DRR performs well in simulation 1 and 2, and shows slightly lower power for the major QTNs than FastRR. However, DRR loses power in detecting QTNs with minor effects, and this difference becomes more and more obvious with the increase of the polygenic background. Among three regression analysis methods, the FastRR performs well in the simulation experiment and has the highest statistical power.

Currently, the two-stage methodologies (Tamba et al., 2017; Zhang et al., 2017; Wen et al., 2018) are more popular in GWAS, which are the alternative approaches to solve the “big P, small N” problem. The FASTmrEMMA (Wen et al., 2018; Wen et al., 2020) algorithm is a fast and accurate two-stage methodology for QTNs detection. We further compare the FastRR and FASTmrEMMA algorithm in this study. The results of simulation experiment 1 and 2 are listed in Supplementary Tables 1, 2A–C. Observably, the FastRR and FASTmrEMMA algorithm are powerful in QTNs detection from the perspective of statistical power. However, the estimation of FASTmrEMMA is slightly worse than FastRR, which has a relatively larger MSE. In addition, FASTmrEMMA consumes a median computing time (∼150 s for each replication) among all methods, and much more than FastRR. Therefore, the FastRR algorithm was shown to be a good alternative method for multi-locus GWAS.

Mixed linear model methodologies are mainstream in GWAS, most of them treat QTN effects as fixed effects. In this study, the QTN effects are viewed as random, and it is more consistent with genetic mechanisms (Wen et al., 2018). In order to avoid the influence of the increase of computational complexity, several acceleration techniques have been incorporated into the algorithm. Firstly, we estimate and fix the polygenic-to-residual variance ratio, and then transform the phenotypes and genotypes in the first stage. This technique was adopted in pLARmEB (Zhang et al., 2017) and FASTmrEMMA (Wen et al., 2018), avoiding re-estimating this ratio for each marker. Secondly, the marginal correlation in the second step is similar to the single marker scanning, which quickly filters the unassociated SNPs. The number of SNPs reduces from tens of thousands to hundreds of putative QTNs in the simulation and real data analysis. Thirdly, in the multi-locus model (6), we assume
all $\sigma^2 = \phi^2$, thus only two variance components ($\phi^2$ and $\sigma^2$) requires DRR to estimate. The results from simulation and real data analysis indicate that the estimation under this simple assumption has achieved better performance for QTN detection and fast computational speed. Lastly, multithreaded marginal correlation is implemented in the FastRR.

Efficient mixed model association and DEMMA as popular single-locus genome scan approaches have been successfully used in GWAS to dissect quantitative traits. However, single-locus approaches ignore the potential information of neighboring markers and fail to consider the joint minor effect of multiple genetic markers on a trait. The FastRR algorithm overcomes this shortcoming. From the results of the simulation, FastRR is more powerful in the detection of QTNs (Figures 2, 3). Although the three popular variable selection approaches, lasso, adaptive lasso, and SCAD, utilize the potential information of markers, the detection and estimation are not accurate (Tables 1, 2A–C). This may be due to the over shrinkage of QTNs, and therefore the effect of QTN is smaller than the true effect; specifically, the minor effect of QTN is shrunk to 0. Consequently, the FastRR algorithm is shown to be more robust in data analysis.

The analysis of large-scale genetic data in GWAS is a hot topic at present. In this study, the correlation coefficients are employed to reduce the dimension of potentially related variables, which are then included in the subsequent multi-locus analysis. The threshold of the correlation coefficient test is set to 0.01 (Tamba et al., 2017), and even the slight correlations between predictors and the response are easily captured. The other thresholds are used, such as 0.001 and 0.0001, which are more rigorous and allows the filtering out of the minor effect loci that will not be included in the multi-locus model. The threshold equal to 0.05 is too loose and includes a large number of SNPs over the threshold; the putative loci are included in the subsequent multi-locus analysis, and furthermore, it is time consuming and results in intractable calculations. Thus, it is reasonable to choose 0.01 as the threshold value in the selection of variables.

**DATA AVAILABILITY STATEMENT**

The rice data used for the analysis described in this manuscript was obtained from https://doi.org/10.1093/bioinformatics/btaa345; The Arabidopsis data used for the analysis described in this manuscript was obtained from http://www.arabidopsis.usc.edu/.

**AUTHOR CONTRIBUTIONS**

JZ and JC conceived and supervised the study. JZ, MC, YW, and YL performed all experiments and analyzed the data and revised the manuscript. YZ, MC, SW, and JC made all figures and forms. JZ, YW, and JC also wrote and revised the manuscript. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2021.649196/full#supplementary-material

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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