Mining Favorable Alleles for Rice Coleoptile Elongation Length Sensitivity to Exogenous Gibberellin Under Submergence Condition

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Abstract
High sensitivity of rice coleoptile elongation length to exogenous gibberellin is a beneficial trait to utilize superior rice cultivars that could not be used originally under water direct-seeded conditions. In the present study, we mined favorable alleles for the trait by combining the phenotypic data of 358 rice accessions with their genotype data of 262 simple sequence repeat (SSR) markers via genome wide association mapping method. Totally, 17 SSR marker loci significantly associated with gibberellin sensitivity index (GSI) of coleoptile elongation length under 10 cm depth of water, were detected by general linear model and mixed linear model across two years, with percent phenotypic variation explained larger than 10%. Twenty nine favorable alleles for GSI on the 17 loci were discovered with phenotypic effect value (PEV) larger than 0.1 cm/cm and RM6869-110 bp showed the largest PEV (0.27 cm/cm). Based on PEV of marker-alleles having positive effects on GSI, seven parental combinations were predicted to improve GSI. In addition, 7 loci for GSI were co-located with loci associated with coleoptile elongation length per se, and one locus (RM1182 on chromosome 5) was co-located with that associated with coleoptile elongation length after gibberellin-soaked seed, under germination condition of 10 cm depth of water. These favorable allele(s) could be used to improve two target traits simultaneously.

Keywords Rice · Favorable alleles · Coleoptile elongation · Anoxia · Gibberellic acid · Water direct-seeded rice · Association mapping

Introduction
Rice (Oryza sativa L.) is the most important cereal crop in the world. Due to a lack of manpower and higher wages, rice growers turn to the direct seeding method (Angaji et al. 2010). Direct-seeded rice is a common production method in southern Louisiana and areas in Texas and California State, USA (Hardke and Scott 2013). In the same time, rice plants suffer from submergence (flooding) and poor seedling establishment.

Flooding is one of the serious problems which affect rice production in South and Southeast Asia, where the majority of the world's rice is grown, about 20 million hectares of rice land is prone to flooding. Flooding creates hypoxic or anoxic condition resulting in poor germination and seedling establishment, even in some cases leads to plant death within few days of full submergence (IRRI 2016; Singh et al. 2017). There are different categories of flooding; we are interested in submergence during germination also known as anaerobic germination. On this condition, rapid seedling elongation can provide successful establishment, and escape...
from submergence stress, hence provides required oxygen for normal growth.

For successful establishment and escape from submergence stress, priming technique is involved to enhance the start of germination processes (Silva and Silva 2016). Doley et al. (2018) studied priming effect on 243 rice genotypes for anaerobic germination under 10 cm of flooding. They found that priming rice seeds for 24 h with different solutions enhanced anaerobic germination under flooding compared to control. In addition, priming three rice cultivars for 48 h was the best seed invigoration treatment under well watered condition (Mulbah and Adjety 2018). Furthermore, Sarkar 2012 studied two near isogenic lines under flooding and non-flooding conditions. His result revealed that seed priming improved the seedling establishment under anaerobic conditions. Recently, it was observed that rice seed priming followed by sun drying can improve anaerobic germination (Senapati et al., 2019).

Angaji et al. (2010) identified a few tolerant genotypes of over 8000 genotypes screened for the tolerance of flooding during germination. Under submergence, successful rice coleoptile elongation depends on hydrolases induction to mobilize endosperm; α-amylases play a central role in this process. Gibberellic acid (GA3) is an important hormone induces α-amylases expression resulting in germination and seedling growth in rice under anaerobic conditions (Lee et al. 2014). Kaneko et al. (2002) also found that active GA3 is important for α-amylases expression in rice endosperm. Moreover, in barley the expression of the α-amylase gene is up-regulated by exogenous GA3 (Gubler et al. 2002). Rice cultivars have different sensitivity to exogenous gibberellin concentrations via seed treatment, reflecting upon seedling performance (Guadagnin et al. 2017). Likewise, a study on rice showed that the most effective concentration was 2000 ppm GA3, which enhances seedlings length of BW196 (Mutinda et al. 2017).

Mining favorable alleles for coleoptile length (CL), coleoptile length gibberellic acid sensitivity (CLGS) and its gibberellic acid sensitivity index (GSI) for water direct-seeded rice would provide breeders to improve traits. In 2004, Jiang et al. (2004) detected five QTLs for anoxia germinability from 81 RILs with phenotypic variation ranged from 10.5 to 19.6% on chromosomes 1, 2, 5 and 7, respectively. Furthermore, they detected three pairs of epistasis loci located on chromosomes 2, 3, 5 and 11 with significant effects ranging from 16.7 to 48.8%. Five putative QTLs controlling flooding tolerance during germination in rice were detected on chromosomes 1, 3, 7 and 9, explaining 17.9–33.5% of the phenotypic variation (Angaji et al. 2010). Septiningsih et al. (2013) identified six QTLs of mapping 175 $F_{2:3}$ families genotypes, using 118 SSR markers, on chromosomes 2, 5, 6 and 7 associated with a survival rate of seedling under 10 cm depth of water. Baltzar et al. (2014) detected two major QTLs associated with the survival rate of seedling while analysis 300 lines $F_{2:3}$ derived from the cross of IR64 and the aus landrace Nanhi. One QTL derived from Nanhi detected on chromosome 7 explained 22.3% phenotypic variance, while the other one was detected on chromosome 2 from IR64 with increased effect. Recently, three QTLs associated with anaerobic germination detected by analysis 285 $F_{2:3}$ genotypes derived from a cross between Tai Nguyen and Anda using 6 K SNP chip (Kim and Reinke 2018). Two QTLs were detected on chromosome 1 and one QTL on chromosome 8 with variance explained percentage ranged from 5.49 to 14.14%. Taking all together, the QTLs reported up to now for anoxic (flooding) conditions are 4, 4, 2, 3, 1, 6, 1, 1, and 1 on chromosomes 1, 2, 3, 5, 6, 7, 8, 9 and 11 respectively.

In our study, two points are new compared with previous research. One is the QTL detection method (we use GWAS for this trait), the other is gibberellin-treated seeds and germinated under 10 cm depth of water. It is the first report mined favorable alleles of coleoptile elongation and its sensitivity to gibberellic acid for water direct-seeded rice by association mapping using 262 SSR markers from the natural population The aims of the present study were to (1) investigate the phenotypic variation of CL, CLGS and GSI under anoxic condition; (2) identify QTLs and mine the favorable alleles for CL, CLGS and GSI by genome-wide association mapping; (3) predict parental combinations for improve CLGS with high GSI according to superior accessions screened in this study.

Materials and Methods

Plant Materials

The seeds of the 358 rice genotypes were collected, stored, and supplied by State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, China (Supplementary Table 1).

Field Planting

All the seeds of the tested materials were sowed in the seedling nursery of paddy field in Jiangpu Experimental Station, Nanjing Agricultural University, in mid-May and transplanted in mid-June in 2017. The experiment was evaluated in a randomized block design with three replications. All the recommended package of practices was followed. In 2018, the dates of sowing and transplanting and field managements were equivalent to 2017. The purpose of field planting was to harvest fresh seeds for germination experiments.
Evaluation of Coleoptile Elongation Length and Its Sensitivity to Gibberellic Acid Under 10 cm Depth of Water Condition

Fifty seeds of each accession were used for each treatment (0 ppm-GA3 and 2000 ppm-GA3). Under the control treatment, the seeds were soaked in distilled water for 24 h; while under GA3 treatment, the seeds were soaked in GA3 solution (2000 ppm) for 24 h. Thirty uniformed soaked seed were visually selected out of the 50 and transferred to a paper towel, lined up on 3 cm from the lower edge, covered with two layers of moist filter paper and rolled the paper up, sailed with a rubber band and placed vertically in plastic box (44 cm × 31 cm × 15 cm) and submerged under 10 cm depth water. The plastic boxes were put under the natural conditions for 13 days to allow the seeds germinate and grow (Supplementary Fig. 1). On the fourteenth day, the coleoptile elongation lengths of 10 seedlings in each replicate of each treatment in each accession were measured with a ruler, and recorded as CL (cm) for the distilled water treatment and CLGS (cm) for the GA3 treatment. Coleoptile elongation length sensitivity of an accession to GA3 was designated as gibberellin sensitivity index (GSI) and was determined using the following formulas:

\[
GSI(cm/cm) = \frac{CLGS - CL}{CL}
\]

where CLGS is coleoptile elongation length (cm) under GA3 treatment, and CL is coleoptile elongation length (cm) under distilled water treatment (control).

Phenotypic Data Analysis

The mean, standard deviation, maximum, minimum and coefficient of variation for the CL and CLGS trait were calculated by using XLSTAT: Statistical software for Excel (Version 20.6.5) available from https://www.xlstat.com/en/. Microsoft Excel software 2016 was used to compute the broad-sense heritability using the following formula (Wang et al. 2007):

\[
H^2_B = \frac{\delta^2_g}{\delta^2_g + \delta^2_e/n}
\]

where \(\delta^2_g\) is genetic variance, \(\delta^2_e\) is error variance, and \(n\) is a number of replicates.

The correlation coefficient was calculated between each of CL, CLGS and GSI by using SPSS statistics 19 (Weaver and Wuensch 2013).

SSR Marker Genotyping

Based on the existing data published on rice molecular mapping, as well as microsatellite data (Temnykh et al. 2000; McCouch et al. 2002; Varshney et al. 2005), 262 pairs of SSR primers distributed on the 12 chromosomes of rice were utilized in genotyping. Leaf blade tissue of a single individual plant in each accession was used to extract genomic DNA using the method described by Dang et al. (2019). DNA amplification primers were synthesized by Shanghai Generay Biotech Co. Ltd., Shanghai, China. Every 10 µl PCR mixture contained 1 µl genomic DNA, 0.7 µl of the forward primer and the same amount (0.7 µl) of reverse primer, 10 × Buffer (free MgCl2) 1 µl, dNTPs 0.2 µl, 0.1 µl of Taq polymerase, 0.6 µl MgCl2, and 5.7 µl ddH2O. PCR amplification was performed on a Peltier Thermal Cycler (PTC-100™, MJ Research™ Incorporated, USA) under denaturation of 94°C for 5 min; 34 cycles of denaturation at 94°C for 30 s, annealing at 55 ~ 61°C (depending on the primer used) for 1 min, with extension at 72°C for 1 min, and, finally, an extension at 72°C for 10 min. Visualization of the resultant PCR products was done on an 8% polyacrylamide gel run for 1 h at 150 V and observed through silver staining.

Population Genetic Structure Analysis

Using STRUCTURE version 2.2 (Falush et al. 2007) the genetic clusters in the 358 accessions were identified. A mean log-likelihood value over five runs set each K (K from 2 to 10) with random starting points. The length of the burn-in period was set to 50,000 iterations and defined a run of 100,000 Markov Chain Monte Carlo (MCMC) replicates after burn-in was used. If the mean log-likelihood value was positively correlated with the model parameter K; a suitable value for K could not be determined. In this situation, the optimal K value was determined through an ad hoc statistic (ΔK) based on the rate of change in [LnP (D)] between successive K values (Evanno et al. 2005). Non-admixed individuals in each genetic group were determined using a Q-matrix assignment greater than 0.9. Power Marker version 3.25 (Liu and Muse 2005) was used to determine the number of alleles per locus, major allele frequency, genetic diversity per locus, and polymorphism information content (PIC) values. The genetic distance was calculated based on 262 molecular markers using Nei’s distance (Nei et al. 1982) and phylogenetic reconstruction was performed using a neighbor-joining method as implemented in Power Marker with the tree viewed using MEGA 4.0 (Tamura et al. 2007). Locus-by-locus analysis of molecular variance (AMOVA) (Weir and Cockerham 1984) based on genetic groups delimited by the Bayesian clustering method in the program Arlequin 3.5 (Excoffier and Lischer 2010) was performed to statistically verify the geographical structure using
SSR and standard multi-locus frequency data. The genetic differentiation coefficient or fixation index \( (F_{st}) \) between subpopulations was calculated using the method proposed by Weir and Hill (2002). The calculation process was performed in Arlequin 3.5 software.

**Linkage Disequilibrium Analysis**

To evaluate the linkage disequilibrium (LD) level, TASSEL 2.1 (Bradbury et al. 2007) software was used in which each pair of SSR loci was evaluated, in all rice accessions and clusters arising from STRUCTURE analysis. The \( D' \) value was used to measure the degree of LD between sites (non-alleles). The formula for calculating the \( D' \) value is given as (Hedrick 1987):

\[
D' = \sum_{i=1}^{u} \sum_{j=1}^{v} p_i q_j |D'_{ij}|
\]

where \( u \) and \( v \) represent the number of alleles of the two loci, \( p_i \) and \( q_j \) the frequency of the \( i \)-th allele at position A and the frequency of the \( j \)-th allele at position B, respectively. \( |D'_{ij}| \) is the absolute value of Lewontin’s (1964) normalized measure \( D'_{ij} \).

\[
D'_{ij} = \frac{D_{ij}}{D_{ij}^{\text{max}}}
\]

where \( D_{ij}^{\text{max}} \) is the maximum amount of disequilibrium possible between the \( i \)-th allele at locus A and the \( j \)-th allele at locus B.

**Genome Wide Association Mapping**

Genome wide association mapping using General Linear Model (GLM, Q) and Mixed Linear Model (MLM, Q+K) was performed using TASSEL 3.0 to calculate the associations between the target trait and markers (Bradbury et al. 2007). The Q matrix was obtained from the analysis results of Structure 2.2, and genetic relatedness (K) matrix was obtained by the software TASSEL 3.0. A false discovery rate (FDR) of 0.001 was used as a threshold for multiple testing according to the correction method published by Benjamini and Hochberg (1995). In this study, marker loci with phenotypic variation explained (PVE) > 7% were considered for further analysis. The phenotypic effect values of the alleles amplified were calculated based on the null allele (not amplified) method described by Breseghello and Sorrells (2006).

**Results**

**Phenotypic Variations of CL, CLGS and GSI**

The phenotypic data of the CL and CLGS followed a normal distribution as showed in Fig. 1, which is also confirmed by Kurtosis and Skewness values for both years (Table 1). The mean value for CL over 358 accessions was 2.59 cm with a range from 0.82 to 3.82 cm in 2017. The coefficient of variance was 20.62% and broad sense heritability was 98.50%. In 2018, the results for CL were similar to those of the previous year (Table 1). On the other hand, the mean value for CLGS was 3.04 cm with a range from 1.25 to 4.76 cm in 2017. The coefficient of variation was 19.61% with \( H^2_b \) of 95.92%. Also, the results for CLGS obtained in 2018 were similar to those of the previous year (Table 1). The broad-sense heritability for CL and CLGS was higher than 90% in both years, indicating that the phenotypic variations of the two traits were mainly controlled by genetic factors.

Gibberellic acid treatment increased the coleoptile elongation length by 0.45 cm/cm and 0.46 cm/cm averaged over 358 accessions in 2017 and 2018, respectively, compared with those of water treatment. The GSI ranged from 0 to 2.12 cm/cm in 2017, while the range in 2018 was from 0 to 2.39 cm/cm (Fig. 2), indicating there exist variations in coleoptile elongation length sensitivity to GA3 among the 358 genotypes used. According to the performances of both CL and GSI grown in the 10 cm depth of water, 6 accessions were considered as superior germplasms for water direct-seeded rice (Table 2). The most sensitive accession to GA3 is Gaoliangqing with GSI of 2.26 cm/cm, followed by Wuxiangjing14 (0.91 cm/cm), Changdaotou (0.87 cm/cm), Hongdao35 (0.79 cm/cm), Zhenghan2 (0.71 cm/cm) and Huajing5 (0.61 cm/cm).

Figure 3 shows the difference in coleoptile elongation length between 0 ppm-GA3 treatment and 2000 ppm-GA3 treatment under 10 cm depth of water in accessions Changdaotou. It can be seen from Fig. 3 that the deference between CL and CLGS are clear.

The correlation coefficients between CL, CLGS and GSI are presented in Table 3. The result revealed positive and highly significant between CLGS and GSI. While the correlation coefficient between CL and GSI was negative and highly significant.

**Genetic Diversity of the Entire Population Revealed by SSR Markers**

The genetic diversity of the 358 accessions was determined using 262 SSR markers distributed on the 12 chromosomes in rice. Totally 2474 marker alleles were identified with average of 9.443 alleles per locus (ranged from 2 to 25)
The gene diversity value averaged over 262 loci was 0.731 with a range from 0.100 (RM7163 on chromosome 11) to 0.937 (RM7545 on chromosome 10). The polymorphic information content (PIC) value averaged over 262 loci was 0.702 with a range from 0.095 (RM7163 on chromosome 11) to 0.933 (RM7545 on chromosome 10).

(Supplementary Table 2).
While 33 markers showed PIC value less than 0.5, the PIC value of 92 markers were more or equal 0.8, and 137 markers were in between 0.5 and 0.8. These results indicate high genetic diversity in the population used.

**Population Genetic Structure**

Genetic structure analysis of the entire populations showed an increase in likelihood function \( \ln P(\text{K}) \) value with the increase of subpopulations (Supplementary Fig. 2a). Supplementary Fig. 2b shows that \( \Delta K \) value reached maximum at \( K = 6 \). Therefore, the entire population can be divided into 6 sub-populations. A neighbour-joining tree of the 358 accessions was constructed based on Nei’s genetic distance (Supplementary Fig. 2d), and the results were consistent with the results from the Structure analysis. Using the criterion of \( Q \) value > 0.9, each accession was sorted into the corresponding subpopulation. 325 accessions entered into 6 subpopulations (known as SP1, SP2, SP3, SP4, SP5 and SP6) (Supplementary Fig. 2), and the remaining 33 accessions entered into an admixture subpopulation. The numbers of accessions SP1, SP2, SP3, SP4, SP5 and SP6 were 52, 75, 38, 24, 70 and 66, respectively (Supplementary Table 1).

By checking the resources of the 358 accessions, it was found that the 6 subpopulations divided above had different geographic origins or ecotypes. Accessions in SP1 were all from Vietnam (Indica rice). SP2 contains accessions from middle China and a few numbers of northeast accessions (Temperate japonica). Most of the accessions in SP3 are modern cultivars bred in the north-central of Jiangsu province (Temperate japonica). SP4 has accessions from middle-east China (Temperate japonica). SP5 accessions were mainly from south Jiangsu province (Temperate japonica) and SP6 had tall, late-maturing accessions and a small number of northeast accessions in the Taihu Lake Basin (Temperate japonica), as showed in Supplementary Table 1.

The results of the analysis of molecular variance (AMOVA) indicated that 46.2% of the total genetic variation occurred between the subpopulations, whereas 53.8% occurred within the subpopulations (Table 4). These results indicate a high degree of genetic differentiation across the six subpopulations.

### Genetic Diversity of the Six Subpopulations

The basic genetic information of each subpopulation is shown in Table 5. SP6 has the highest number of alleles per locus (4.057), the highest genetic diversity (0.524), while SP3 has the lowest numbers of alleles per locus (2.031), the lowest genetic diversity (0.276), among the 6 subpopulations (Table 5). Compared with the entire population, the genetic parameters of each subpopulation were significantly different.
reduced, indicating that the alleles of partial loci were fixed during the process of differentiation of each subpopulation.

### Pairwise Fst Values and Nei’s Genetic Distance Among the Subpopulations

The $F_{st}$ values, which reflected the genetic differentiation extent between two subpopulations, for the 15 pairs of subpopulations are shown below the diagonal (Table 6). The $F_{st}$ value between SP2 and SP5 was the lowest (0.376), while that between SP3 and SP4 was the highest (0.632). Nei’s genetic distance between SP2 and SP5 was short (0.528), while the distance between SP3 and SP4 was long (0.771) (Table 6). The results in Table 6 indicate that the pairwise $F_{st}$ value can reflect the genetic distance between subpopulations.

#### Table 4 Analysis of molecular variance (AMOVA) for 6 subpopulation of rice varieties

| Source of variance       | DF  | Sum of squares | Variance components | Percentage of variation | $P$ value |
|-------------------------|-----|----------------|---------------------|-------------------------|-----------|
| Among populations       | 5   | 7264.41        | 26.86               | 46.16                   | <0.01     |
| Among individuals within populations | 319 | 9993.67        | 31.33               | 53.84                   | <0.01     |
| Total                   | 324 | 17258.08       | 58.18               |                         |           |

*DF* degree of freedoms

#### Table 5 Summary statistics for each subpopulation

| Subpopulation | Sample size | Alleles | Alleles/locus | Genetic diversity | PIC |
|---------------|-------------|---------|---------------|-------------------|-----|
| SP1           | 52          | 720     | 2.748         | 0.407             | 0.360 |
| SP2           | 75          | 1060    | 4.046         | 0.506             | 0.457 |
| SP3           | 38          | 532     | 2.031         | 0.276             | 0.240 |
| SP4           | 24          | 555     | 2.118         | 0.320             | 0.276 |
| SP5           | 70          | 919     | 3.508         | 0.434             | 0.834 |
| SP6           | 66          | 1063    | 4.057         | 0.524             | 0.477 |
| Total         | 325         | 4849    | 18.508        | 2.467             | 2.644 |

*PIC* the polymorphism information content, *SP* subpopulation

#### Table 6 Pairwise estimates of $F_{st}$ and Nei’s genetic distance among the 6 subpopulations

|            | SP1 | SP2 | SP3 | SP4 | SP5 | SP6 |
|------------|-----|-----|-----|-----|-----|-----|
| SP1        | –   | 0.608 | 0.655 | 0.714 | 0.576 | 0.703 |
| SP2        | 0.429 | –   | 0.549 | 0.672 | 0.528 | 0.607 |
| SP3        | 0.577 | 0.435 | –   | 0.690 | 0.487 | 0.717 |
| SP4        | 0.567 | 0.457 | 0.632 | –   | 0.556 | 0.771 |
| SP5        | 0.471 | 0.376 | 0.444 | 0.484 | –   | 0.672 |
| SP6        | 0.474 | 0.392 | 0.524 | 0.487 | 0.454 | –   |

Nei’s genetic distance estimates appear above the diagonal and pairwise $F_{st}$ value appears below the diagonal. All the $F_{st}$ values are significant at $\alpha=0.01$ probability level.

*SP* subpopulation
accessions in SP2 have been subjected to extreme artificial selection.

**SSR Marker Loci Associated with CL, Favorable Alleles and Their Carrier Accessions**

Twenty three marker loci were detected using the GLM model and two SSR loci were detected using MLM model in both years with PVE more than 7% (one SSR marker locus common between the two models). All markers were distributed on all chromosomes except chromosome 5 and chromosome 7 (Table 8). The range of PVE was from 7.19% (RM1013 on chromosome 9) to 18.22% (RM6327 on chromosome 11) in 2017 and the results were similar in 2018.

Table 9 shows the top 39 positive favorable alleles of the significant association loci with PEV more than 0.5 cm and

| Table 7 Comparison of the D' value of LD for pairwise loci in all subpopulations |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sub-populations                | No. of LD locus pairs | Ratio (%) | Frequency of D' value (P < 0.01) | Mean of D' |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SP1                             | 299             | 0.87            | 0               | 56              | 112             | 93              | 38              | 0.58            |
| SP2                             | 880             | 2.57            | 0               | 149             | 320             | 281             | 130             | 0.59            |
| SP3                             | 135             | 0.39            | 8               | 19              | 37              | 46              | 24              | 0.58            |
| SP4                             | 57              | 0.17            | 0               | 12              | 17              | 13              | 15              | 0.61            |
| SP5                             | 661             | 1.93            | 34              | 114             | 204             | 222             | 87              | 0.57            |
| SP6                             | 1139            | 3.33            | 26              | 184             | 423             | 366             | 140             | 0.57            |

LD linkage disequilibrium, Ratio: between the number of significant LD locus pairs and total number of locus pairs.

Table 8 SSR marker loci associated significantly (FDR = 0.001) with CL in 358 rice accessions

| Marker* | Ch | Position (cM) | P value 2017 | P value 2018 | R² or PVE (%) 2017 | PVE (%) 2018 | FDR 2017 | FDR 2018 |
|---------|----|---------------|---------------|---------------|---------------------|---------------|----------|----------|
| RM1231  | 1  | 98.5          | 7.3E–05       | 2.9E–05       | 11.27               | 11.78         | 7.1E–05  | 2.8E–05  |
| RM128   | 1  | 123.2         | 6.2E–04       | 7.2E–04       | 9.30                | 9.11          | 6.2E–04  | 7.1E–04  |
| RM6831  | 1  | 157.6         | 6.4E–04       | 8.5E–04       | 9.46                | 9.19          | 4.79E–04 | 7.46E–04 |
| RM5340  | 2  | 36.3          | 9.9E–04       | 3.2E–04       | 10.87               | 11.76         | 9.9E–04  | 3.2E–04  |
| RM5356  | 2  | 43.3          | 1.7E–05       | 4.1E–05       | 10.83               | 10.12         | 1.4E–05  | 4.0E–05  |
| RM1358  | 2  | 48.1          | 4.2E–05       | 9.1E–06       | 10.63               | 11.56         | 4.0E–05  | 6.4E–06  |
| RM300   | 2  | 54.6          | 2.6E–04       | 3.2E–05       | 11.01               | 12.40         | 2.6E–04  | 3.1E–05  |
| RM106   | 2  | 101.5         | 5.0E–04       | 1.0E–04       | 12.98               | 14.24         | 5.0E–04  | 1.0E–04  |
| RM489   | 3  | 20.3          | 4.1E–04       | 4.1E–05       | 10.42               | 12.11         | 4.1E–04  | 4.0E–05  |
| RM3766  | 3  | 34.8          | 1.6E–04       | 2.0E–05       | 9.79                | 11.12         | 1.6E–04  | 1.8E–05  |
| RM7197  | 3  | 44.4          | 8.3E–05       | 3.7E–05       | 10.31               | 10.78         | 8.1E–05  | 3.5E–05  |
| RM232   | 3  | 76.7          | 3.6E–04       | 4.2E–04       | 10.69               | 10.40         | 3.6E–04  | 4.2E–04  |
| RM3513  | 3  | 99.6          | 5.4E–04       | 3.5E–04       | 8.24                | 8.47          | 5.3E–04  | 3.5E–04  |
| RM7563  | 4  | 68.3          | 2.3E–05       | 9.4E–06       | 8.82                | 9.28          | 2.1E–05  | 7.1E–06  |
| RM3836  | 4  | 108.2         | 4.3E–04       | 5.1E–04       | 9.27                | 9.09          | 4.3E–04  | 5.1E–04  |
| RM508   | 6  | 2.3           | 6.2E–05       | 5.6E–06       | 10.95               | 12.44         | 5.9E–05  | 2.5E–06  |
| RM3754  | 8  | 112.6         | 9.8E–05       | 6.1E–05       | 8.62                | 8.90          | 9.7E–05  | 6.0E–05  |
| RM3533  | 9  | 65.1          | 3.4E–04       | 3.8E–04       | 10.32               | 10.15         | 3.3E–04  | 3.8E–04  |
| RM5384  | 9  | 90.7          | 6.3E–05       | 1.7E–05       | 10.31               | 11.14         | 6.2E–05  | 1.5E–05  |
| RM1013  | 9  | 93.5          | 2.4E–04       | 1.6E–04       | 7.19                | 7.40          | 2.4E–04  | 1.6E–04  |
| RM269   | 10 | 69.6          | 2.1E–05       | 1.1E–05       | 8.18                | 8.54          | 1.8E–05  | 8.8E–06  |
| RM286   | 11 | 0.1           | 5.4E–04       | 2.6E–04       | 7.82                | 8.24          | 5.4E–04  | 2.6E–04  |
| RM6327  | 11 | 1.7           | 1.0E–03       | 2.1E–04       | 18.22               | 19.96         | 1.0E–03  | 2.1E–04  |
| RM6296  | 12 | 26.7          | 1.2E–04       | 2.1E–05       | 6.98                | 8.00          | 1.2E–04  | 1.9E–05  |
| RM6296  | 12 | 26.7          | 9.82E–04      | 6.16E–04      | 7.64                | 8.04          | 8.70E–04 | 4.49E–04 |

*Markers in bold are novel markers detected in current study; markers in italic detected by MLM model

Ch. Chromosome, cM Centimorgan, PVE phenotypic variation explained, FDR False discovery rate
their typical carrier materials (which carrying the desired alleles with the highest phenotypic value) for CL in both years. The PEV for those alleles ranged from 0.504 cm of RM3754-80pb (typical carrier accession Songjing12) to 1.594 cm of RM6327-215pb (typical carrier accession Wanqu429).

| Locus- allele | Ch | Phenotypic effect value | Typical carrier |
|--------------|----|------------------------|-----------------|
| RM1231-150   | 1  | 0.752                  | Haidongqing     |
| RM1231-170   | 1  | 0.735                  | Haidongqing     |
| RM1231-185   | 1  | 0.671                  | Longdao4        |
| RM128-160    | 1  | 0.546                  | Haidongqing     |
| RM6831-150   | 1  | 0.527                  | Haidongqing     |
| RM6831-150   | 1  | 0.527                  | Baishidao       |
| RM5340-145   | 2  | 0.865                  | Haidongqing     |
| RM5356-155   | 2  | 0.617                  | Haidongqing     |
| RM1358-175   | 2  | 0.563                  | Longdao4        |
| RM300-140    | 2  | 0.517                  | Haidongqing     |
| RM106-180    | 2  | 1.081                  | Haidongqing     |
| RM106-255    | 2  | 0.592                  | Longdao4        |
| RM106-260    | 2  | 0.758                  | Muzhan4         |
| RM106-280    | 2  | 0.824                  | Longdao4        |
| RM106-290    | 2  | 0.764                  | Chushuhuang     |
| RM106-305    | 2  | 0.713                  | Huangkewanguangtou |
| RM106-330    | 2  | 0.796                  | Wanqu429        |
| RM489-170    | 3  | 0.552                  | Nannongjing003  |
| RM489-175    | 3  | 0.934                  | Haidongqing     |
| RM489-185    | 3  | 0.697                  | Muzhan4         |
| RM489-240    | 3  | 0.536                  | Longdao4        |
| RM489-300    | 3  | 0.541                  | Huangkewanguangtou |
| RM7197-155   | 3  | 0.550                  | Haidongqing     |
| RM232-150    | 3  | 0.870                  | Haidongqing     |
| RM232-160    | 3  | 0.549                  | Haidongqing     |
| RM3513-125   | 3  | 0.499                  | Longdao4        |
| RM3513-80    | 3  | 0.616                  | Haidongqing     |
| RM3836-115   | 4  | 0.670                  | Haidongqing     |
| RM508-270    | 6  | 0.538                  | Muzhan4         |
| RM3754-80    | 8  | 0.515                  | Songjing12      |
| RM5384-160   | 9  | 0.538                  | Haidongqing     |
| RM6327-120   | 11 | 1.274                  | Xiangjing9407   |
| RM6327-180   | 11 | 1.412                  | Yue98 (49.1)    |
| RM6327-185   | 11 | 0.911                  | Cai             |
| RM6327-195   | 11 | 1.440                  | Nannongjing003  |
| RM6327-200   | 11 | 1.438                  | Longdao4        |
| RM6327-210   | 11 | 1.499                  | Si4161          |
| RM6327-215   | 11 | 1.609                  | Wanqu429        |
| RM6327-230   | 11 | 1.583                  | Huangkewanguangtou |

| Marker locus | Ch | 2017 | 2018 | Mean |
|--------------|----|------|------|------|
| RM1231-150   | 1  | 0.4864 | −0.2151 | 0.5005 |
| RM128-160    | 1  | 0.2742 | −0.2750 | 0.2747 |
| RM6831-150   | 1  | 0.3960 | −0.3040 | 0.3900 |
| RM5340-145   | 2  | 0.4499 | −0.0409 | 0.4045 |
| RM5356-155   | 2  | 0.2963 | −0.2987 | 0.2975 |
| RM1358-175   | 2  | 0.3713 | −0.3750 | 0.3730 |
| RM300-140    | 2  | 0.3499 | −0.0400 | 0.3563 |
| RM106-180    | 2  | 0.6270 | −0.6244 | 0.6244 |
| RM489-170    | 3  | 0.5109 | −0.1292 | 0.3957 |
| RM3766-150   | 3  | 0.2829 | −0.3257 | 0.2828 |
| RM7197-150   | 3  | 0.1942 | −0.4940 | 0.1933 |
| RM232-150    | 3  | 0.5038 | −0.1312 | 0.5044 |
| RM3513-125   | 3  | 0.5027 | −0.5050 | 0.5027 |
| RM7563-150   | 4  | 0.1552 | −0.0802 | 0.1528 |
| RM3836-115   | 4  | 0.3677 | −0.3491 | 0.3627 |
| RM508-140    | 6  | 0.2672 | −0.4483 | 0.2667 |
| RM3754-140   | 8  | 0.2947 | −0.3999 | 0.2933 |
| RM3533-140   | 9  | 0.2852 | −0.3891 | 0.2836 |
| RM5384-160   | 9  | 0.2930 | −0.0889 | 0.2925 |
| RM1013-140   | 9  | 0.2080 | −0.1323 | 0.2298 |
| RM269-150    | 10 | 0.2962 | −0.4102 | 0.2950 |
| RM286-150    | 11 | 0.1698 | −0.1373 | 0.1717 |
| RM6327-150   | 11 | 1.3959 | −1.4013 | 1.4013 |
| RM6296-150   | 12 | 0.2110 | −0.3508 | 0.2234 |

Six marker loci showed positive average allele effect (AAE+), without negative allele effect (PVE more than 7%); RM6327 was the highest with AAE+ equal to 1.396 cm, followed by RM106 with AAE+ 0.627 cm, RM3513 with AAE+ 0.503 cm, RM1358 with AAE+ 0.371 cm, RM5356 with AAE+ 0.296 cm and RM128 with AAE+ 0.274 cm (Table 10). Based on phenotypic effect value of marker-alleles which have positive effect on CL, the best parental combinations were selected from the top 20 accessions. Seven parental combinations predicted to improve CL; and the predicted phenotypic effect ranged from 0.850 cm to 0.940 cm (Table 11).

### SSR Marker Loci Associated with CLGS, Favorable Alleles and Their Carrier Accessions

Twenty-one SSR loci for CLGS were detected using GLM mode and two SSR loci using MLM model in both years with PVE more than 7% (one SSR marker locus common between the two models). Overall, 22 SSR markers were distributed on all chromosomes except chromosome 12.
The range of PVE was from 7.06% (RM3688 on chromosome 2) to 17.24% (RM3773 on chromosome 10) in 2017 and the results were similar in 2018.

Table 13 shows the top 56 positive favorable alleles of the significant association loci with the PEV more than 0.5 cm (PVE more than 7%) and their typical carrier accessions for CLGS. The PEV for those alleles ranged from 1.087 cm for RM562-180 (typical carrier accession Xiaoqingmang) to 0.506 cm for RM283-150 (typical carrier accession Zhengan2).

Three markers showed positive average allele effect (AAE), without negative allele effect (PVE more than 7%); RM562 was the highest one with AAE 0.721 cm, followed by RM434 with AAE 0.538 cm and RM3453 with AAE 0.494 cm (Table 14).

Comparing the association analysis for CL and CLGS, the result showed that RM3754 (chromosome 8) was

| Trait     | Parental combination | Mean coleoptile length (cm) | No. of positive alleles for parents | No. of positive alleles predicted | Predicted increase of phenotypic effect value (cm) | Predict coleoptile length (cm) |
|-----------|----------------------|-----------------------------|-----------------------------------|----------------------------------|-----------------------------------------------|-------------------------------|
| CL        | Haidongqing × Yue98  | 3.572                       | 18 × 15                           | 19                               | 0.940                                         | 4.511                         |
| CL        | Haidongqing × Longdao4 | 3.744                      | 18 × 15                           | 19                               | 0.850                                         | 4.594                         |
| CL        | Longdao4 × Wanzhognqiu| 3.568                       | 15 × 18                           | 19                               | 0.850                                         | 4.418                         |
| CL        | Longdao4 × Xiaobaidao | 3.560                       | 15 × 18                           | 19                               | 0.850                                         | 4.410                         |
| CL        | Tiekwanguangtou × Yue98 | 3.448                     | 18 × 15                           | 19                               | 0.940                                         | 4.387                         |
| CL        | Wanzhognqiu × Yue98  | 3.395                       | 18 × 15                           | 19                               | 0.934                                         | 4.329                         |
| CL        | Xiaobaidao × Yue98   | 3.388                       | 18 × 15                           | 19                               | 0.940                                         | 4.327                         |

CL coleoptile length in without treatment under 10 cm depth of water

### Table 12

SSR marker loci associated significantly (FDR = 0.001) with CLGS

| Marker* | Ch | Position (cM) | P value   | R² (%) or PVE | FDR  |
|---------|----|---------------|-----------|---------------|------|
|         |    |               | 2017      | 2018          | 2017 | 2018 |
| RM283   | 1  | 19.9          | 5.7E-05   | 2.7E-05       | 5.6E-05 | 2.5E-05 |
| RM3453  | 1  | 25.4          | 4.3E-04   | 4.5E-04       | 8.76  | 8.86  |
| RM562   | 1  | 65.4          | 4.3E-04   | 2.5E-04       | 12.83 | 13.67 |
| RM14    | 1  | 181.8         | 1.4E-05   | 1.3E-05       | 8.94  | 9.10  |
| RM3688  | 2  | 88.2          | 2.2E-04   | 1.5E-04       | 7.06  | 7.40  |
| RM471   | 4  | 53.8          | 2.7E-05   | 2.5E-05       | 8.90  | 9.09  |
| RM6114  | 4  | 72            | 4.8E-04   | 2.6E-04       | 7.28  | 7.77  |
| RM6589  | 4  | 85.2          | 1.8E-04   | 9.8E-05       | 11.60 | 12.17 |
| RM1182  | 5  | 3             | 1.3E-05   | 8.2E-06       | 8.38  | 8.78  |
| RM5818  | 5  | 144.9         | 9.8E-05   | 1.1E-04       | 7.87  | 7.93  |
| RM510   | 6  | 11.5          | 5.3E-04   | 3.1E-04       | 8.09  | 8.57  |
| RM3330  | 6  | 61.6          | 1.5E-04   | 8.6E-05       | 8.84  | 9.32  |
| RM7309  | 6  | 100.3         | 2.6E-04   | 2.6E-04       | 8.06  | 8.21  |
| RM3138  | 6  | 110.6         | 5.8E-04   | 1.9E-04       | 7.17  | 7.94  |
| RM3589  | 7  | 89.8          | 3.4E-04   | 3.5E-04       | 7.96  | 8.07  |
| RM134   | 7  | 99.6          | 2.2E-04   | 1.6E-04       | 7.30  | 7.62  |
| RM1306  | 7  | 116.1         | 2.6E-04   | 1.9E-04       | 8.91  | 9.25  |
| RM8243  | 8  | 50.8          | 4.6E-05   | 4.4E-05       | 7.69  | 7.85  |
| RM3754  | 8  | 112.6         | 1.4E-05   | 9.4E-06       | 7.91  | 8.25  |
| RM434   | 9  | 57.7          | 2.8E-04   | 2.9E-04       | 8.46  | 8.62  |
| RM3773  | 10 | 58.9          | 3.3E-05   | 2.5E-05       | 11.47 | 11.77 |
| RM3773  | 10 | 58.9          | 2.2E-04   | 2.2E-04       | 17.24 | 17.23 |
| RM7170  | 11 | 101.9         | 3.4E-04   | 2.3E-04       | 7.33  | 7.68  |

*Markers in bold are novel markers detected in current study; markers in italic detected by MLM model

Ch. Chromosome, cM Centimorgan, PVE phenotypic variation explained, FDR false discovery rate
co-associated with both CL and CLGS analysis with PVE 8.62% and 7.91% for CL and CLGS in 2017, respectively; and the result was similar in 2018. RM3754-80 bp shows phenotypic effect value 0.504 cm for CL and the typical carrier is Songjing12. While RM3754-100 bp shows PEV 0.550 cm for CLGS and the typical carrier is Changdaotou, and RM3754-85 bp shows PEV 0.519 cm and typical carrier Zhenghan2. In 2017, positive AAE value of RM3754 was
0.155 cm and 0.211 cm for CL and CLGS, respectively; and the result was similar in 2018.

Based on PEV of marker-alleles which have positive effects, the best parental combinations were selected from the top 20 accessions for CLGS (Table 15). Seven parental combinations were predicted to improve CLGS ranged from 0.814 to 0.922 cm. Among all, Changdaotou and Hongdao35 were selected before as superior accessions.

### Table 15 Parental combination, coleoptile length, number of alleles and predicted phenotypic effect value (CLGS)

| Trait  | Parental combination       | Mean coleoptile length (cm) | No. of positive alleles for parents | No. of positive alleles predicted | Predicted increase of phenotypic effect value (cm) | Predict coleoptile length (cm) |
|--------|---------------------------|----------------------------|--------------------------------------|----------------------------------|-----------------------------------------------|-------------------------------|
| CLGS   | Changdaotou × Maijieqing  | 4.413                      | 20×22                                | 22                               | 0.913                                         | 5.325                         |
|        | Changdaotou × Daliangdao  | 4.246                      | 20×22                                | 22                               | 0.913                                         | 5.159                         |
|        | Changdaotou × Qiyunuo10   | 4.239                      | 20×21                                | 22                               | 0.814                                         | 5.053                         |
|        | Changdaotou × Hongdao35   | 4.214                      | 20×22                                | 22                               | 0.867                                         | 5.080                         |
|        | Changdaotou × Xiganggu    | 4.214                      | 20×21                                | 22                               | 0.865                                         | 5.079                         |
|        | Maijieqing × Shuaishaban  | 4.299                      | 22×21                                | 22                               | 0.922                                         | 5.221                         |
|        | Shuaishaban × Daliangdao  | 4.133                      | 21×22                                | 22                               | 0.922                                         | 5.055                         |

*CLGS coleoptile length with GA3 treatment under 10 cm depth of water*

### SSR Marker Loci Associated with GSI, Favorable Alleles and Their Carrier Accessions

Seventeen SSR loci for GSI were detected using GLM and MLM model in years 2017 and in 2018 with PVE more than 10%. The 17 SSR marker loci were distributed on all chromosomes except chromosome 7 and 8 (Table 16). The range of PVE was from 10.19% (RM112 on chromosome 2) to 19.69% (RM162 on chromosome 6).

| Marker* | Ch | Position (cM) | P Value | R2 (%) or PVE | FDR |
|---------|----|---------------|---------|---------------|-----|
| RM128   | 1  | 123.2         | 8.86E–05| 3.41E–05      | 14.44 | 15.33 | 8.35E–05 | 1.53E–05 |
| RM297   | 1  | 126.5         | 4.54E–05| 1.08E–04      | 36.69 | 34.72 | 3.97E–05 | 3.47E–04 |
| RM1358  | 2  | 48.1          | 3.96E–05| 1.93E–05      | 14.41 | 14.96 | 3.86E–05 | 1.71E–05 |
| RM300   | 2  | 54.6          | 5.03E–05| 5.23E–05      | 16.16 | 16.03 | 4.95E–05 | 5.09E–05 |
| RM112   | 2  | 137.5         | 5.00E–04| 1.63E–04      | 10.19 | 11.37 | 4.97E–04 | 1.14E–04 |
| RM3766  | 3  | 34.8          | 1.71E–05| 4.81E–05      | 15.02 | 14.02 | 1.60E–05 | 4.66E–05 |
| RM3766  | 3  | 34.8          | 1.03E–04| 2.65E–04      | 13.64 | 12.75 | 9.81E–05 | 1.27E–04 |
| RM3513  | 3  | 99.6          | 5.13E–05| 4.56E–05      | 13.36 | 13.36 | 5.04E–05 | 4.40E–05 |
| RM3836  | 4  | 108.2         | 1.06E–04| 9.24E–04      | 13.57 | 13.61 | 1.05E–04 | 9.12E–05 |
| RM1182  | 5  | 3             | 6.03E–06| 3.48E–06      | 14.37 | 14.74 | 4.55E–06 | 5.06E–07 |
| RM162   | 6  | 114.9         | 2.25E–05| 1.98E–05      | 19.69 | 19.69 | 2.15E–05 | 1.77E–05 |
| RM162   | 6  | 114.9         | 7.22E–04| 5.18E–04      | 16.45 | 16.81 | 7.19E–04 | 1.68E–04 |
| RM20    | 9  | 81.2          | 2.21E–05| 2.88E–05      | 15.49 | 15.12 | 2.11E–05 | 2.7E–05  |
| RM20    | 9  | 81.2          | 4.13E–05| 2.55E–04      | 15.07 | 13.24 | 3.49E–05 | 1.32E–04 |
| RM311   | 10 | 25.2          | 1.98E–06| 2.11E–05      | 12.70 | 10.75 | 2.74E–07 | 1.92E–05 |
| RM1125  | 10 | 46.8          | 1.13E–04| 7.41E–05      | 16.09 | 16.47 | 1.12E–04 | 7.28E–05 |
| RM269   | 10 | 69.6          | 1.27E–05| 4.41E–05      | 11.37 | 10.28 | 1.15E–05 | 4.24E–05 |
| RM269   | 10 | 69.6          | 2.05E–05| 1.67E–05      | 11.14 | 11.31 | 1.30E–05 | 1.13E–05 |
| RM304   | 10 | 73            | 1.22E–05| 2.02E–05      | 14.68 | 14.06 | 1.09E–05 | 1.82E–05 |
| RM304   | 10 | 73            | 1.31E–04| 3.17E–04      | 12.42 | 11.50 | 1.27E–04 | 1.15E–05 |
| RM21    | 11 | 85.7          | 3.23E–04| 4.79E–05      | 13.85 | 15.86 | 3.23E–04 | 4.64E–05 |
| RM21    | 11 | 85.7          | 1.12E–04| 2.25E–05      | 15.69 | 17.51 | 1.08E–04 | 1.75E–05 |
| RM6869  | 12 | 75.8          | 6.14E–06| 6.89E–06      | 18.51 | 18.31 | 4.75E–06 | 4.33E–06 |
| RM6869  | 12 | 75.8          | 7.96E–04| 7.72E–04      | 14.51 | 14.55 | 7.94E–04 | 1.45E–04 |

*Markers in bold are novel markers detected in current study; markers in italic detected by MLM model
Ch chromosome, cM Centimorgan, PVE phenotypic variation explained, FDR false discovery rate
2) to 36.69% (RM297 on chromosome 1) in 2017 and the results were similar in 2018.

Table 17 shows the top 29 positive favorable alleles of the significant association loci with PEV more than 0.1 cm/cm and their typical carrier accessions for GSI in years 2017 and in 2018. The PEV for those alleles ranged from 0.100 cm/cm for RM6869-110pb (typical carrier accession Yangdao) to 0.270 cm/cm for RM6869-110pb (typical carrier accession Yangdao).

Two markers showed positive average allele effect (AAE), without negative allele effect (PVE more than 10%); RM304 was the highest one with AAE 0.212 cm/cm, followed by RM297 with AAE 0.105 cm/cm. Among 17 markers, the marker RM304 was showing the highest positively AAE (Table 18).

### Table 17: Top 29 positive favorable alleles, phenotypic effect value and typical carrier materials for GSI

| Locus- allele | Ch | Phenotypic effect value | Typical carrier |
|---------------|----|-------------------------|-----------------|
| RM128-170     | 1  | 0.267                   | 0.269           | Yangdao         |
| RM128-185     | 1  | 0.220                   | 0.189           | Wuxiangjing14   |
| RM297-180     | 1  | 0.213                   | 0.214           | Hongdao35       |
| RM1358-170    | 2  | 0.106                   | 0.096           | Zhenghan2       |
| RM1358-180    | 2  | 0.116                   | 0.127           | Gaoliangqing    |
| RM300-125     | 2  | 0.148                   | 0.142           | Puxiadaotou     |
| RM300-130     | 2  | 0.139                   | 0.133           | Yilimang        |
| RM112-120     | 2  | 0.182                   | 0.196           | Gaoliangqing    |
| RM112-125     | 2  | 0.243                   | 0.236           | Yangdao         |
| RM3766-145    | 3  | 0.120                   | 0.125           | Gaoliangqing    |
| RM3513-80     | 3  | 0.103                   | 0.105           | Gaoliangqing    |
| RM3836-125    | 4  | 0.130                   | 0.127           | Yangdao         |
| RM1182-165    | 5  | 0.120                   | 0.093           | Wuxiangjing14   |
| RM162-210     | 6  | 0.171                   | 0.183           | Gaoliangqing    |
| RM162-220     | 6  | 0.227                   | 0.220           | Yangdao         |
| RM162-300     | 6  | 0.185                   | 0.188           | Hongdao35       |
| RM20-160      | 9  | 0.114                   | 0.116           | Gaoliangqing    |
| RM20-210      | 9  | 0.185                   | 0.181           | Wuxiangjing14   |
| RM311-150     | 10 | 0.147                   | 0.137           | Wuxiangjing14   |
| RM311-155     | 10 | 0.138                   | 0.143           | Yanjing9        |
| RM1125-165    | 10 | 0.258                   | 0.226           | Sanjiang2       |
| RM269-165     | 10 | 0.139                   | 0.147           | Gaoliangqing    |
| RM269-180     | 10 | 0.183                   | 0.170           | Sanjiang2       |
| RM304-135     | 10 | 0.212                   | 0.208           | Gaoliangqing    |
| RM21-130      | 11 | 0.108                   | 0.110           | Gaoliangqing    |
| RM21-135      | 11 | 0.131                   | 0.129           | Wuxiangjing14   |
| RM6869-110    | 12 | 0.268                   | 0.272           | Yangdao         |
| RM6869-125    | 12 | 0.094                   | 0.106           | Gaoliangqing    |
| RM6869-130    | 12 | 0.170                   | 0.139           | Gaidaoqing      |

### Ch. chromosome, AAE average allele effect

The results showed that 7 markers were associated with both GSI and CL traits. Among all positive favorable alleles, RM3513-80 bp shows phenotypic effect value 0.616 cm for CL and the typical carrier is Haidongqing, and the same marker allele shows phenotypic effect value 0.104 cm/cm for GSI and the typical carrier is Gaoliangqing.

Furthermore, we found that RM1182 was associated with both GSI and CLGS traits. Among all positive alleles, RM1182-145 bp showed phenotypic effect value 0.729 cm for CLGS and the typical carrier was Maijieqing. RM1182-150 bp showed phenotypic effect value 0.578 cm for CLGS and the typical carrier was Shuangchengnuo. While RM1182-165 bp showed phenotypic effect value 0.106 cm/cm for GSI and the typical carrier was Wuxiangjing14.

Based on PEV of marker-alleles which have positive effects on GSI, the best parental combinations were selected from the top 20 accessions for GSI. Seven parental combinations were predicted to improve GSI from 0.154 to 0.160 cm/cm (Table 19).

Comparing the parental combination accessions which selected for CL, CLGS and GSI, the accession Hongdao35 had been found to share in both CLGS and GSI parental combinations; also it was one of the superior accessions. In addition to, all parental combination accessions selected were temperate japonica; and these accessions were categorized under three subpopulations Sp2, Sp3 and Sp5.
Discussion

Treating the seeds with gibberellic acid can enhance the coleoptile elongation length under submergence condition, which is considered as key of survival under anoxic conditions for water direct-seeded rice (Gubler et al. 2002; Kaneko et al. 2002; Lee et al. 2014; Mutinda et al. 2017).

In this study, there were great variations in the traits under investigation. The mean value for CL ranged from 0.82 to 3.82 cm in 2017, as well as 2018, the same result was obtained by Hsu and Tung (2015). While mean value for CLGS ranged from 1.25 to 4.76 cm 2017 and the results were similar in 2018, which is consistent with the results of others (Guadagnin et al. 2017; Mutinda et al. 2017). Furthermore, there was a positive correlation between CLGS with GSI, moreover, a wide range for GSI indicating the existence of genotype sensitivity. Hence, the broad-sense heritability was higher than 90% in both years for CL and CLGS, which means that the genetic effect is mainly controlling both CL and CLGS comparing to the environmental effect (Visscher et al. 2008).

The six superior accessions were found in this study belonging to temperate japonica. Previous studies reported that coleoptile performance of temperate japonica varieties (as sub-species of japonica varieties) was better than indica varieties under anaerobic conditions (Lasanthi-Kudahettige et al. 2007; Hsu and Tung 2015). The AMOVA results (46.16% genetic variability among subpopulations and 53.84% within subpopulations) revealed that the rice genotypes under our study highly variable and suitable for conducting association mapping as demonstrated in previous studies (Adeyemo et al. 2005; Agrama and Eizenga 2008; Jaiswal et al. 2012; Bergamaschi and Lama 2015). These accessions probably had a complex breeding history involving intercrossing and introgression between germplasm from diverse backgrounds, overlaid with strong selection pressure for agronomic and quality characteristics (Mather et al. 2004).

In association mapping, the LD used is present in the germplasm set under study. As well, LD might not only be influenced by recombination but also by various other forces (Flint-Garcia et al. 2003). Contrasting to the previous studies, LD was decaying in our study at more than 70 cM, this can be attributed due to outcrossing and recombination events that have been used in breeding programs (Garris et al. 2003; Lu et al. 2005; Olsen et al. 2006; Dang et al. 2014).

Association mapping is a very prevalent method for the explanation of the genetic basis of complex traits in plants. Different statistical approaches had been designed to deal with the superior marker-phenotype association that could be caused by the population structure. GLM depends only on Q matrix generated during the study of population structure while MLM accounts for both population structure and the kinship. Generally GLM will detect a higher number of significant marker-trait associations than in MLM; while MLM is more accurate in claiming associations than GLM (Korte and Farlow 2013). QQ plots (GLM & MLM) were generated to demonstrate that population structure is only controlling the confounding factors that could bias the results (Wei et al 2017) as shown in Fig. 4.

For coleoptile elongation length under control treatment (CL), RM6327 on chromosome11 explained the maximum phenotypic variation, 16 accessions out of 358 (4.47%) showed an excellent alleles RM6327-215 bp, with the largest phenotypic effect values (1.609 cm in 2017 and 1.578 cm in 2018) and the typical carrier accession was Wanqu428.

Exogenous GA3 plays an important role in rice coleoptile elongation under submergence, anoxia or hypoxia (Kotanoguchi et al. 2008). In this study, for CLGS, RM 562 on chromosome 1 explained the maximum phenotypic value, 14 accessions out of 358 (3.92%) possess the excellent alleles RM562-180 bp; with the largest phenotypic effect (1.087 cm in both 2017 and 2018) and the typical carrier accession was Xiaoqingmang.

Table 19 Parental combination, coleoptile length, number of alleles and predicted phenotypic effect value (GSI)

| Trait | Parental combination | Mean coleoptile length (cm/cm) | No. of positive alleles for parents | No. of positive alleles predicted | Predicted increase of phenotypic effect value (cm/cm) | Predict coleoptile length (cm/cm) |
|-------|----------------------|-------------------------------|-----------------------------------|---------------------------------|-------------------------------------------------------|-----------------------------------|
| GSI   | Gaidaoqing × Hongdao35 | 0.912                         | 16 × 14                           | 17                              | 0.157                                                 | 1.069                             |
|       | Baishuqing × Hongdao35 | 0.912                         | 15 × 14                           | 17                              | 0.154                                                 | 1.066                             |
|       | Yilimang × Hongdao35   | 0.872                         | 16 × 14                           | 17                              | 0.160                                                 | 1.032                             |
|       | Yanjing9 × Hongdao35   | 0.823                         | 15 × 14                           | 17                              | 0.159                                                 | 0.982                             |
|       | Hongdao35 × Sishitou   | 0.789                         | 14 × 15                           | 17                              | 0.154                                                 | 0.942                             |
|       | Hongdao35 × Baikewandao| 0.736                         | 14 × 16                           | 17                              | 0.157                                                 | 0.893                             |
|       | Hongdao35 × Yebaidao   | 0.704                         | 14 × 15                           | 17                              | 0.154                                                 | 0.858                             |

GSI gibberellic acid sensitivity index
The differences detected between GSI and CLGS verified that they are functioned differently (Zhang et al. 2017). This result indicated that GSI explained the different genetic mechanisms of coleoptile treated with GA3 under anoxic condition. Additionally, RM297 was detected with the highest PVE (36.69% and 34.72% in 2017 and in 2018, respectively), indicated that this was chromosome segment controlling GSI and considered as a promising marker which can increase GSI. Wang et al. (2012) and Zhao et al. (2017) had been detected similar results with PVE value exceeded 20%.

Pleiotropy is the well-established phenomenon of a single gene affecting multiple traits. It has long played a central role in theoretical, experimental, and clinical research in genetics, development, molecular biology, evolution, and medicine (Paaby and Rockman 2012). Seven markers were detected in this study to have a pleiotropic effect for CL and CLGS. A similar result had been found in wheat by Chai et al. (2019); while Ookawa et al. (2010) used the pleiotropy phenomenon for improving rice lodging resistance and yield.

Taking all together, 24, 22 and 17 marker loci were associated significantly with CL, CLGS and GSI. Among all, 34 marker loci were novel in current study; the rest (20) of marker loci were detected in previous studies (Itoh et al. 2001; Kikuchi et al. 2003; Sakai et al. 2003; Toojinda et al. 2003; Huang et al. 2003; Jan et al. 2004; Ling et al. 2004; Angaji 2008; Lo et al. 2008; Narsai et al. 2009; Angaji et al. 2010; Magome et al. 2013; Septiningsih et al. 2013; Hsu and Tung 2015; Tomita and Ishii 2018).

Improving rice coleoptile length under anaerobic condition, all favorable alleles might be pyramided as much as possible into one variety. Crosses between accessions which have favorable alleles (as hybridization parents) should improve target trait. Pyramiding best favorable alleles into new cultivar might need multi round crossing (Cheng et al. 2015). The results of this study provided basic marker
information and accession information for breeding cultivars suitable for anaerobic conditions (water direct-seeded rice).

In conclusion, there is a phenotypic variation for coleoptile length under control treatment (CL), coleoptile length under GA3 treatment (CLGS) and molecular marker allele diversity among 358 accessions. Twenty four markers loci significantly associated with CL and 22 markers loci associated with CLGS (PVE > 10%). Thirty nine favorable alleles for CL and 56 favorable alleles for CLGS (PEV > 0.5 cm) were detected across two years by GLM and MLM analysis models. While, 17 markers loci significantly associated with GSI (PVE > 10%), with 29 favorable alleles were detected across two years by GLM and MLM analysis models. Twelve, thirteen and twenty three typical carrier accessions for CL, CLGS and GSI, respectively, possessing the favorable alleles could be used to improve those traits under anoxic condition.

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Author contributions DH, DA designed the research; DA, NA and MSE carried out the field experiment; DA, EL and DX carried out the molecular experiment; DA, NA analyzed data; and DA wrote the manuscript; DH revised the manuscript.

Compliance with Ethical Standards

Conflict of interest No conflict of interest among authors and in the research work.

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