Dissection of three quantitative trait loci for grain size on the long arm of chromosome 10 in rice (*Oryza sativa* L.)

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Background. Thousand grain weight is a key component of grain yield in rice, and a trait closely related to grain length and grain width that are important traits for grain quality. Causal genes for 16 quantitative trait loci (QTL) affecting these traits have been cloned, but more QTL remain to be characterized for establishing a genetic regulating network. A QTL controlling grain size in rice, *qGS10*, was previously mapped in the interval RM6100−RM228 on chromosome 10. This study aimed to delimitate this QTL to a more precise location.

Method. A total of 12 populations were used. The ZC9 population comprised 203 $S_{1:2}$ families derived from a residual heterozygous (RH) plant in the F_9 generation of the *indica* rice cross Teqing/IRBB52, segregating the upper region of RM6100−RM228 and three more regions on chromosomes 1, 9 and 11. The Ti52-1 population comprised 171 S_1 plants derived from one RH plant in F_7 of Teqing/IRBB52, segregating a single interval that was in the lower portion of RM6100−RM228. The other ten populations were all derived from Ti52-1, including five S_1 populations with sequential segregating regions covering the target region and five near isogenic line (NIL) populations maintaining the same segregating pattern. QTL analysis for 1000-grain weight, grain length and grain width was performed using QTL IciMapping and SAS procedure GLM.

Result. Three QTL were separated in the original *qGS10* region. The *qGL10.1* was located in the upper region RM6704−RM3773, shown to affect grain length only. The *qGS10.1* was located within a 207.1-kb interval flanked by InDel markers Te20811 and Te21018, having a stable and relatively high effect on all the three traits analyzed. The *qGS10.2* was located within a 1.2-Mb interval flanked by simple sequence repeat markers RM3123 and RM6673. This QTL also affected all the three traits but the effect was inconsistent across different experiments. QTL for grain size were also detected in all the other three segregating regions.

Conclusion. Three QTL for grain size that were tightly linked on the long arm of chromosome 10 of rice were separated using NIL populations with sequential segregating regions. One of them, *qGS10.1*, had a stable and relatively high effect on grain weight, grain length and grain width, providing a good candidate for gene cloning. Another QTL, *qGS10.2*, had a significant effect on all the three traits but the effect was inconsistent across different experiments, providing an example of genotype-by-environmental interaction.

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19 **Abstract**

20 **Background.** Thousand grain weight is a key component of grain yield in rice, and a trait closely

21 related to grain length and grain width that are important traits for grain quality. Causal genes for

22 16 quantitative trait loci (QTL) affecting these traits have been cloned, but more QTL remain to

23 be characterized for establishing a genetic regulating network. A QTL controlling grain size in

24 rice, *qGS10*, was previously mapped in the interval RM6100−RM228 on chromosome 10. This

25 study aimed to delimitate this QTL to a more precise location.

26 **Method.** A total of 12 populations were used. The ZC9 population comprised 203 $S_{1:2}$ families

27 derived from a residual heterozygous (RH) plant in the F_9 generation of the *indica* rice cross

28 Teqing/IRBB52, segregating the upper region of RM6100−RM228 and three more regions on

29 chromosomes 1, 9 and 11. The Ti52-1 population comprised 171 S_1 plants derived from one RH

30 plant in F_7 of Teqing/IRBB52, segregating a single interval that was in the lower portion of

31 RM6100−RM228. The other ten populations were all derived from Ti52-1, including five S_1

32 populations with sequential segregating regions covering the target region and five near isogenic

33 line (NIL) populations maintaining the same segregating pattern. QTL analysis for 1000-grain

34 weight, grain length and grain width was performed using QTL IciMapping and SAS procedure

35 GLM.

36 **Result.** Three QTL were separated in the original *qGS10* region. The *qGL10.1* was located in the

37 upper region RM6704−RM3773, shown to affect grain length only. The *qGS10.1* was located

38 within a 207.1-kb interval flanked by InDel markers Te20811 and Te21018, having a stable and

39 relatively high effect on all the three traits analyzed. The *qGS10.2* was located within a 1.2-Mb

40 interval flanked by simple sequence repeat markers RM3123 and RM6673. This QTL also

41 affected all the three traits but the effect was inconsistent across different experiments. QTL for

42 grain size were also detected in all the other three segregating regions.

43 **Conclusion.** Three QTL for grain size that were tightly linked on the long arm of chromosome

44 10 of rice were separated using NIL populations with sequential segregating regions. One of

45 them, *qGS10.1*, had a stable and relatively high effect on grain weight, grain length and grain

46 width, providing a good candidate for gene cloning. Another QTL, *qGS10.2*, had a significant

47 effect on all the three traits but the effect was inconsistent across different experiments,

48 providing an example of genotype-by-environmental interaction.

49 **Keywords:** Grain weight, Grain size, Tightly-linked, Quantitative trait locus, Rice

INTRODUCTION

Thousand grain weight (TGW) is a key component of grain yield in rice, and a trait closely related to grain length (GL) and grain width (GW) that are important traits for grain quality. These traits are all complex and inherited quantitatively. To date, causal genes for 16 quantitative trait loci (QTL) affecting these traits have been isolated. They are distributed across eight of the 12 chromosomes of rice. Two of them, *GW7/GL7* and *GS9* (*Wang et al., 2015a; Wang et al., 2015b; Zhao et al., 2018*), have large effect on grain shape but no effect on TGW due to opposite allelic directions of the effects on GL and GW. Four of them, *GW2* (*Song et al., 2007*), *GS5* (*Li et al., 2011*), *GSE5* (*Duan et al., 2017*) and *GW8* (*Wang et al., 2012*), have larger effects on TGW and GW than on GL. Six others, *GL2/GS2* (*Che et al., 2016; Hu et al., 2015*), *GS3* (*Fan et al., 2006*), *GL3.1/qGL3* (*Qi et al., 2012; Zhang et al., 2012*), *OsLG3* (*Yu et al., 2017*), *qTGW3* (*Hu et al., 2018*) and *GW6a* (*Song et al., 2015*), have larger effects on TGW and GL than on GW. The remaining four, *OsLG3b* (*Yu et al., 2018*), *GL4* (*Wu et al., 2017*), *TGW6* (*Ishimaru et al., 2013*) and *GLW7* (*Si et al., 2016*), have large effects on TGW and GL but no effect on GW. These genes encode proteins that are involved in regulating cell proliferation and elongation, including phytohormones, transcriptional regulatory factors and components of pathways such as ubiquitin-proteasome, G protein signaling and MAPK signaling (*Li et al., 2018*). These studies have substantially improved our understanding of the molecular basis of grain weight in rice, but the information is rather fragmental and more genes remain to be isolated and characterized (*Zuo & Li, 2014*).

In genetic analysis of agronomic traits, close linkage of QTL controlling the same trait has been frequently observed. Cloned QTL having major effects on grain weight and grain size in rice have provided clear examples. *OsLG3* and *OsLG3b* for grain length (*Yu et al.,* 2017; *Yu et al., 2018*) were located in a 1.7-Mb region of chromosome 3, *GS5* and *GSE5* for grain width in a 1.9-Mb region of chromosome 5 (*Li et al., 2011; Duan et al., 2017*), and *TGW6* and *GW6a* for grain weight in a 0.6-Mb region of chromosome 6 (*Ishimaru et al., 2013; Song et al., 2015*). Similar results have also been reported for QTL having minor effects on these traits. Targeting at a QTL region affecting grain weight difference between two *indcia* rice varieties, six minor-effect QTL were separated in a 7.1-Mb interval on chromosome 1, including *qTGW1.1a*, *qTGW1.1b*, *qTGW1.2a*, *qTGW1.2b*, *qGS1-35.2* and *qGW1-35.5* (*Wang et al., 2015c; Zhang et al., 2016; Dong et al., 2018*). Assumably, validation and dissection of QTL that were previously

81 coarsely mapped could provide a large number of candidates for isolating individual genes

82 controlling grain weight and grain size in rice.

83   Rice is a staple food for about half of the world's population. Large scale cultivation of

84 hybrid rice has made a great contribution to ensuring the food security in China and may make

85 contributions in many other countries as well (*Yuan, 2014*; *Nalley et al., 2016*). Minor-effect

86 QTL is believed to play a critical role in controlling genetic variations of yield traits among

87 commercial rice varieties and the dissection of these QTL would facilitate the development of

88 high-yielding rice varieties (*Kinoshita et al., 2017*). In our previous studies, QTL analysis for

89 grain size was performed using three recombinant inbred line populations constructed from

90 crosses between parental lines of commercial hybrid rice. One QTL, *qGS10* conferring grain size

91 difference between Teqing (TQ) and IRBB lines was mapped in the interval RM6100–RM228

92 on chromosome 10 and then validated using near isogenic lines (NILs) of the same cross (*Wang*

93 *et al., 2017*). In the present study, NIL populations segregated in sequential order around the

94 *qGS10* region were constructed from the TQ/IRBB52 cross and used for QTL analysis. Three

95 tightly-linked QTL were separated. Among them, *qGS10.1* had a stable and relatively high effect

96 on grain weight, grain length and grain width, *qGS10.2* had significant effects on all the three

97 traits but the effects appeared to be greatly influenced by genotype-by-environmental interaction,

98 and *qGL10.1* was shown to affect grain length only.

MATERIALS AND METHODS

99

Rice materials

100

101 A total of 12 populations of rice (*Oryza sativa* L.) were used (Table 1). As illustrated in Fig. 1

102 and described below, these populations were derived from two residual heterozygous (RH)

103 plants of the *indica* rice cross TQ/IRBB52, namely ZC9 and Ti52-1. ZC9 was an F_9 plant

104 carrying four heterozygous segments, including RM6704–RM3773 on chromosome 10 and three

105 others on chromosomes 1, 9 and 11 (Fig. 2). Ti52-1 was an F_7 plant carrying one heterozygous

106 segment, *i.e.*, RM25766–RM228 on chromosome 10. The intervals RM6704–RM3773 and

107 RM25766–RM228 are adjacent and jointly covered the RM6100–RM228 region previously

108 mapped for *qGS10* (*Wang et al., 2017*). Two populations were constructed and used for QTL

109 mapping. One consisted of 203 $S_{1:2}$ families derived from ZC9, and the other comprised 171 S_1

110 plants produced from Ti52-1 (Table 1).

111   The other ten populations used for QTL mapping were all derived from Ti52-1. Five F_9

112   plants with sequential heterozygous regions covering RM25766–RM228 were selected from

113   progenies of Ti52-1. They were selfed to produce five new S_1 populations. The number of plants

114   in each population ranged from 172 to 343 (Table 1). In each population, non-recombinant

115   homozygotes were selected and selfed. Five NIL populations in $F_{10:11}$ were established, in which

116   the numbers of NILs having the same genotype ranged from 28 to 38 (Table 1).

117   **Field trial and phenotypic evaluation**

118   The filed experiments were conducted at the experimental stations of the China National Rice

119   Research Institute located in either Hangzhou, Zhejiang Province, or Lingshui, Hainan Province,

120   China. The plants were grown with 16.7 cm between plants and 26.7 cm between rows.

121   The six S_1 populations were grown without replication and the plants were harvested

122   individually. The remaining six populations, including one $S_{1:2}$ population and five NIL

123   populations, were tested using a randomized complete block design with two replications.

124   Twelve plants per line were planted in each row. The middle five plants were harvested.

125   Measurement of TGW, GL and GW in all the experiments followed the method of *Zhang et al.*

126   *(2016)*.

127   **DNA marker analysis**

128   For the ZC9 population, marker data and genetic maps have been available (*Sun et al., 2018*).

129   For the other populations, a total of 12 DNA markers were used, including five simple sequence

130   repeat (SSR) and seven InDel markers. The SSRs were selected from the Gramene database

131   (www.gramene.org), while the InDels (Table 2) were developed based on the sequence

132   differences between TQ and IRBB52. DNA extraction and PCR amplification followed the

133   method of *Zheng et al. (1995)* and *Chen et al. (1997)*, respectively.

134   **Data analysis**

135   Mapmarker/Exp 3.0 was performed to calculate the genetic distance which was presented in

136   centiMorgan (cM) using the Kosambi function (*Lander et al., 1987*). For the six S_1 populations

137   and the ZC9 $S_{1:2}$ population, QTL analysis was performed with the inclusive composite interval

138   mapping (ICIM) using the BIP (QTL mapping in the biparental populations) functionality of

139   QTL IciMapping 4.1 (*Meng et al., 2015*). *LOD* thresholds were calculated with 1000

140   permutation tests ($P < 0.05$) and used to declare a putative QTL. For the five NIL populations,

141   SAS procedure GLM (*SAS Institute Inc 1999*) was used to determine phenotypic differences in

142   each population. A mixed model GENOTYPE + LINE (GENOTYPE) + REP +

143   GENOTYPE*REP was applied, in which LINE (GENOTYPE) was defined as a random effect

144   and used as the error term to test GENOTYPE differences (*Dai et al., 2008*). Additive effect and

145   the proportion of phenotypic variance explained (R^2) were estimated with the same model. QTL

146   were designated following the rule proposed by *McCouch and CGSNL (2008)*.

147   **RESULTS**

148   **QTL for grain size detected in the ZC9 and Ti52-1 populations**

149   The ZC9 $S_{1:2}$ population was segregated in the interval RM6704–RM3773 that covered the upper

150   portion of the *qGS10* region, and in three other intervals (Fig. 2). All the four regions were found

151   to have significant effects on one or two of the three grain-size traits analyzed (Table 3). In the

152   interval RM6704–RM3773 on chromosome 10, the effects were significant on GL but non-

153   significant on the other two traits. This QTL explained 9.00 % of the phenotypic variance and the

154   IRBB52 allele increased GL by 0.020 mm. The *qGL1* and *qGW1* associated with RM12210 on

155   chromosome 1 had R^2 values of 32.71 and 5.84 %, respectively, with the IRBB52 allele

156   increased GL by 0.041 mm and decreased GW by 0.007 mm. The *qTGW9* and *qGW9* in the

157   interval RM219–RM1896 on chromosome 9 had R^2 values of 19.15 and 7.42 %, respectively,

158   with the IRBB52 allele increased TGW by 0.16 g and GW by 0.007 mm. The *qTGW11* and

159   *qGW11* in the interval RM224–RM5926 on chromosome 11 had R^2 values of 13.81 and 31.69 %,

160   respectively, with the IRBB52 allele decreased TGW by 0.14 g and GW by 0.016 mm.

161   The Ti52-1 population was segregating in the interval RM25766–RM228 only, which

162   covered the lower portion of the *qGS10* region (Fig. 2). This region was found to have significant

163   effects on all the three grain-size traits analyzed (Table 3). The QTL effects contributed 20.15–

164   36.58 % to the phenotypic variance of each trait, with the TQ allele increasing TGW, GL and

165   GW by 0.59 g, 0.081 mm and 0.024 mm, respectively. Since *qGS10* was previously reported to

166   affect all these three traits with the TQ allele always increasing trait values (*Wang et al., 2017*), it

167   is concluded that *qGS10* was located in the segregating region of the Ti52-1 population, and

168   another QTL, *qGL10.1*, was located in an adjacent interval that was segregated in the ZC9

169   population (Fig. 3A).

170   **Dissection of *qGS10* into two QTL for grain size and grain weight**

171   For further validation and dissection of *qGS10,* five S_1 populations with sequential segregating

172   regions covering the interval RM25766–RM228 were established (Table 1; Fig. 3B). Results of

173   QTL analysis for TGW, GL and GW using these populations are shown in Table 4. Significant

174   QTL effects were detected in G10-2, G10-4 and G10-5 but not in the other two populations. In

175   G10-2, the effects were significant for all the three traits. The TQ allele increased TGW, GL and

176   GW by 0.19 g, 0.030 mm and 0.004 mm, respectively. In G10-4 and G10-5, significant QTL

177   effects were detected for GL only. The enhancing alleles were derived from IRBB52 in both

178   populations, with similar additive effects of 0.019 and 0.023 mm, and similar R^2 values of 5.11

179   and 3.01 %. As shown in Fig. 3B, the region segregated in G10-2 was separated from those

180   segregated in G10-4 and G10-5, indicating that at least two QTL were responsible for the grain-

181   size variations in these populations. One QTL was located in the segregating region of G10-2

182   and another QTL may be located in the common segregating region of G10-4 and G10-5. To

183   validate these results, non-recombinant homozygotes of the five S_1 populations were identified,

184   from which five NIL populations maintaining the original sequential segregating regions were

185   constructed (Table 1; Fig. 3B).

186   Distributions of TGW, GL and GW in the five NIL populations are shown in Fig. 4. The

187   three traits were continuously distributed in all the populations, but differentiation between the

188   two genotypic groups was observed in four populations. Concentration of the TQ and IRBB52

189   homozygous lines respectively in the high- and low-value regions was evident for TGW and GL

190   in G11-2, and for all the three traits in G11-3. The same tendency, though less obvious, was

191   found for TGW and GW in G11-4 and G11-5. Since the segregating region of G11-3 covered the

192   segregating regions of G11-2, G11-4 and G11-5 (Fig. 3B), these results suggest that allelic

193   differences of the two QTL, one located in the segregating region of G11-2 and the other in the

194   common segregating region of G11-4 and G11-5, could be the main cause of phenotypic

195   variations in the five NIL populations.

196   Results of two-way ANOVA for TGW, GL and GW using the five NIL populations are

197   presented in Table 5. In G11-1, no significant phenotypic difference was detected between the

198   two genotypic groups, matching well to the detection of no QTL in its origin population G10-1.

199   In G11-2, the two genotypic groups showed significant differences on each trait. The TQ allele

200   increased TGW, GL and GW by 0.21 g, 0.032 mm and 0.004 mm, with R^2 values of 40.58, 28.55

201   and 3.99 %, respectively. These results were in general agreement with QTL detected in the

202   G10-2 population from which G11-2 was originated, confirming that a QTL affecting TGW, GL

203   and GW was located in the segregating region of G10-2 and G11-2. This QTL, designated

204   *qGS10.1*, was mapped within the region flanked by InDel markers Te20811 and Te21018 (Fig.

205 3B), corresponding to a 207.1-kb region in the Nipponbare genome.

206  In G11-4 and G11-5 which were segregated in homozygous regions of G11-2, significant

207 QTL effects were detected for TGW and GW with the TQ allele always increasing the trait

208 values. The additive effects were 0.13 and 0.12 g for TGW, and 0.012 and 0.011 mm for GW.

209 These results indicate that a QTL for TGW and GW was located in the common segregating

210 region of G11-4 and G11-5. This QTL, designated *qGS10.2*, was mapped within the region

211 flanked by SSR markers RM3123 and RM6673 (Fig. 3B), corresponding to a 1.2-Mb region in

212 the Nipponbare genome. It is noted that the effect of *qGS10.2* on TGW and GW was not detected

213 in the G10-4 and G10-5 populations from which G11-4 and G11-5 were originated. For the other

214 trait GL, a small effect with the enhancing allele derived from IRBB52 was detected in G11-5,

215 which was in accordance with the effects detected in G10-4 and G10-5.

216  For the remaining NIL population, G11-3, significant QTL effects with the enhancing allele

217 derived from TQ were detected for all the three traits. The additive effects and R^2 values

218 estimated for TGW and GW were higher in G11-3 than in G11-2, G11-4 and G11-5. This is

219 reasonable because *qGS10.1* and *qGS10.2* have the same allelic direction and the segregating

220 region of G11-3 covered both QTL.

221 **DISCUSSION**

222 Grain yield of F_1 hybrid rice are known to be positively correlated to both the F_1 heterosis and

223 the parental values. Regarding phenotypic performances of the three key yield components, the

224 highest correlation between restorer line (male parent) and F_1 hybrid was found for grain weight

225 (*Xiang et al., 2016*). Identification of QTL responsible for grain weight variation among

226 important restorer lines would provide new information on understanding the genetic basis of

227 grain yield in rice. In this study, QTL analysis using NILs derived from a cross between rice

228 restorer lines TQ and IRBB52 resulted in the dissection of three QTL for grain weight and grain

229 size that were tightly lined on the long arm of chromosome 10, providing new candidates for

230 gene cloning and marker assisted selection.

231  No QTL for grain weight and grain size detected on chromosome 10 have been cloned, but a

232 number of studies (*Ren et al., 2016*; *Qi et al. 2017*; *Okada et al., 2018*) have located QTL for

233 these traits in the *qGS10* region. In addition, this region was associated with grain-size difference

234 not only between restorer lines TQ and IRBB52, but also between Zhenshan 97 and Milyang 46

235 (*Wang et al., 2017*) that were among the most important female and male parents of commercial

236   hybrid rice, respectively. One of the three QTL for grain size separated in this region, *qGS10.1*,

237   was shown to have a stable and relatively high effect on all the three grain-size traits analyzed.

238   Cloning of this QTL may identify an important gene controlling grain yield of modern rice

239   varieties.

240   Genotype-by-environmental interaction is an important factor influencing the performance

241   of complex traits. The effect of another QTL affecting all the three traits in this study, *qGS10.2*,

242   appeared to be inconsistent across different experiments. In the S_1 populations G10-3 and G10-4

243   tested in Lingshui, this QTL showed non-significant effect on TGW and GW (Table 4). In the

244   NIL populations G11-3 and G11-4 tested in Hangzhou, this QTL was detected for TGW and GW

245   with the R^2 values ranging from 10.32 to 24.56 % (Table 5). Since G11-3 and G11-4 were

246   populations consisting of homozygous lines directly produced from G10-3 and G10-4, the

247   discrepancy is unlikely caused by differences on the genetic background. *Okada et al.* (*2018*)

248   also mapped a QTL for grain weight and grain width in this region, which had a large effect in

249   the late-maturing sub-population but showed non-significant effect in the early-maturing sub-

250   population. They inferred that the difference was caused by the temperature difference from

251   flowering to ripening between the two sub-populations, which was higher for the late-maturing

252   sub-population. Similarly, a stronger effect of *OsMADS56/qHd1* on grain weight was found to be

253   associated with a higher air temperature (*Chen et al., 2018*). The average air temperature from

254   flowering to ripening was 23.5°C for G10-3 and G10-4, and 27.2°C for G11-3 and G11-4. This

255   could be the main cause for the inconsistent effect of *GS10.2* observed in the present study.

256   Linkage drag is a problem commonly encountered in the introgression of alien genes. *Suh et*

257   *al. (2011)* reported that lower spikelet fertility was associated with the introduction of the *Bph8*

258   resistance gene from *O. australiensis* into the background of a japonica rice variety. *Yan et al.*

259   (*2014*) found that transformation of the *Bt* resistance gene into *indica* rice restorer line Minghui

260   63 may have negative effects on grain weight and grain size, resulting in lower grain yield. One

261   of the parental lines used in the present study, IRBB52, is a rice restorer line carrying *Xa4* and

262   *Xa21* in the genetic background of IR24. In the ZC9 population, three regions other than the

263   targeting region on chromosome 10 were also segregated. QTL for grain size were detected in all

264   these three regions (Table 3), among them *qTGW11* and *qGW11* were located in a region

265   covering the *Xa4* locus (Fig. 2). The IRBB52 allele decreased TGW and GW by 0.14 g and

266   0.016 mm. These results suggest that linkage drag may have occurred in the introgression of *Xa4*

267 from the donor parent into IR24. Fine mapping of QTL in this region may provide a solution for

268 this problem.

269 **CONCLUSION**

270 Using NIL populations with sequential segregating regions jointly covering the entire interval for

271 QTL *qGS10* mapped previously, three QTL for grain size tightly-linked on the long arm of

272 chromosome 10 were separated. One of them, *qGS10.1*, had a stable and relatively high effect on

273 grain weight, grain length and grain width, providing a good candidate for gene cloning. Another

274 QTL, *qGS10.2*, had a significant effect on all the three traits but the effect was inconsistent

275 across different experiments, providing an example of genotype-by-environmental interaction.

276

277 **ABBREVIATIONS**

278 TGW   1000-grain weight

279 GL   grain length

280 GW   grain width

281 QTL   quantitative trait locus

282 TQ   Teqing

283 NIL   near isogenic line

284 SSR   simple sequence repeat

285 GLM   general linear model

286

287 **ADDITIONAL INFORMATION AND DECLARATIONS**

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Competing Interests

300 The authors declare there are no competing interests.

301

Author Contributions

303 Jieyun Zhuang, Shaoqing Tang and Tongmin Mou conceived and designed the experiments.

304 Yujun Zhu, Zhichao Sun, Xiaojun Niu and Jiezheng Ying performed the experiments.

305 Yujun Zhu, Yeyang Fan and Jieyun Zhuang analyzed the data.

306 Jieyun Zhuang and Yujun Zhu wrote the manuscript.

307 All authors read and approved the final manuscript.

308

Data Availability

310 The following information was supplied regarding data availability:

311 The raw data has been supplied as a Supplemental Information 1 and 2.

312

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Figure 1

Development of the rice populations used in this study. NIL, near isogenic line

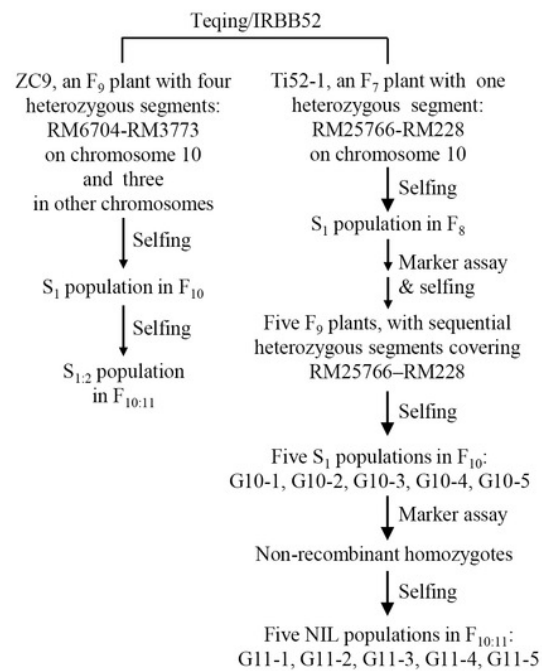



Figure 2

Heterozygous regions of the residual heterozygous plants ZC9 and Ti52-1

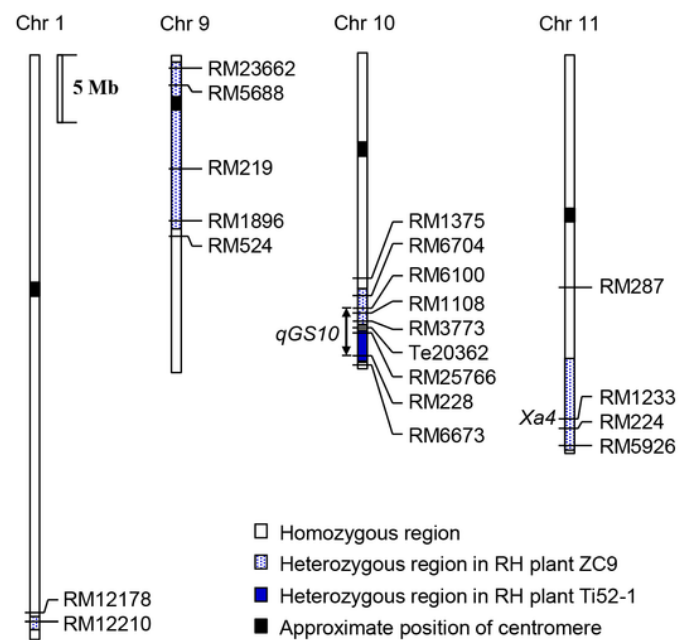



Figure 3

Genotypic composition of the rice populations in the target region on chromosome 10

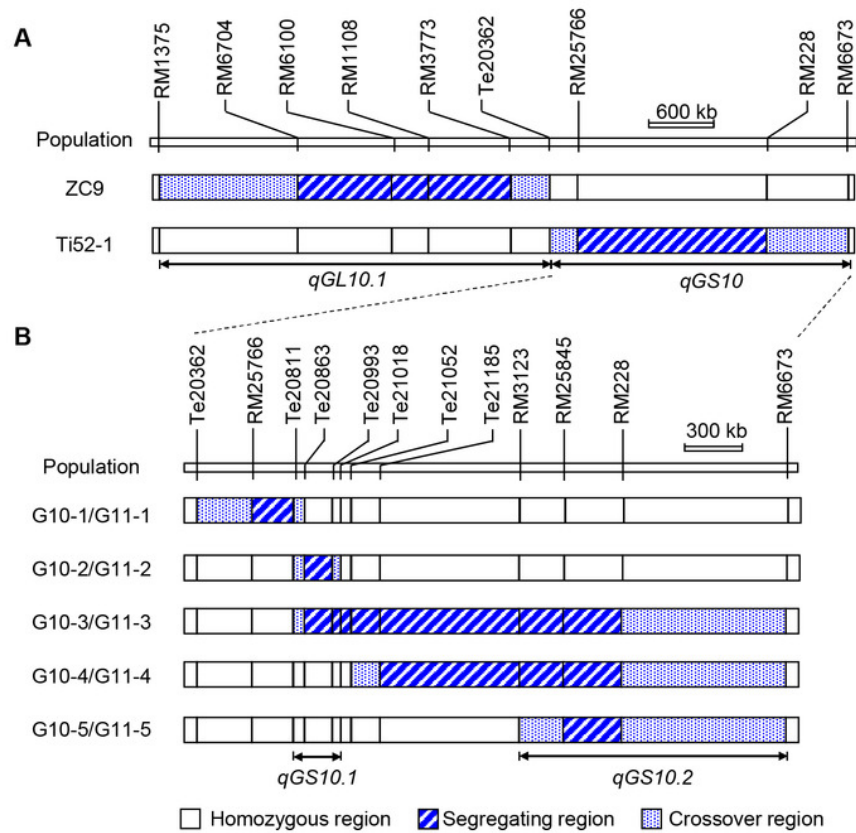



Figure 4

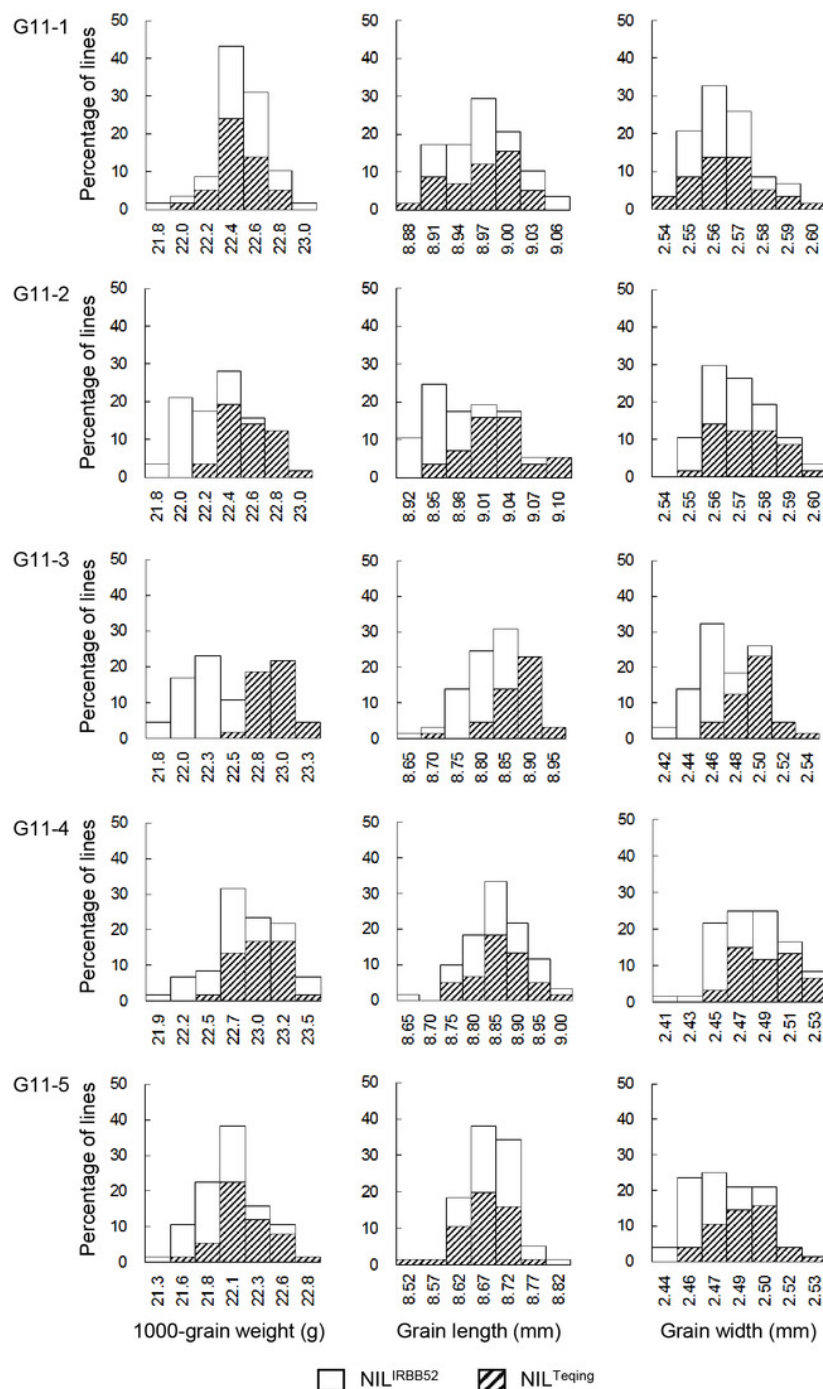
Distributions of 1000-grain weight, grain length and grain width in the five NIL
populations

Rice populations and field experiments

Table 1 Rice populations and field experiments.

Population			Segregating regions on chromosome 10	Sample	Location and growth period
Name	Type	Generation			
For validation of *GS10*					
ZC9	$S_{1:2}$	$F_{10:11}$	RM6704–RM3773	203 lines	HZ: May–Sep. 2017
Ti52-1	S_1	F_8	RM25766–RM228	171 plants	LS: Dec. 2015–Apr. 2016
For dissection of *GS10*					
G10-1	S_1	F_{10}	RM25766–Te20811	278 plants	LS: Dec. 2016–Apr. 2017
G10-2	S_1	F_{10}	Te20863–Te20993	172 plants	LS: Dec. 2016–Apr. 2017
G10-3	S_1	F_{10}	Te20863–RM228	289 plants	LS: Dec. 2016–Apr. 2017
G10-4	S_1	F_{10}	Te21185–RM228	292 plants	LS: Dec. 2016–Apr. 2017
G10-5	S_1	F_{10}	RM25845–RM228	343 plants	LS: Dec. 2016–Apr. 2017
G11-1	NIL	$F_{10:11}$	RM25766–Te20811	29 : 29 lines	HZ: May–Sep. 2017
G11-2	NIL	$F_{10:11}$	Te20863–Te20993	29 : 28 lines	HZ: May–Sep. 2017
G11-3	NIL	$F_{10:11}$	Te20863–RM228	30 : 35 lines	HZ: May–Sep. 2017
G11-4	NIL	$F_{10:11}$	Te21185–RM228	30 : 30 lines	HZ: May–Sep. 2017
G11-5	NIL	$F_{10:11}$	RM25845–RM228	38 : 38 lines	HZ: May–Sep. 2017

Notes.

NIL, near isogenic line; HZ, Hangzhou, Zhejiang Province; LS, Lingshui, Hainan Province. For the five NIL populations, numbers of NILs carrying the Teqing and IRBB52 homozygous segments are indicated before and after the colon, respectively.

Polymorphic markers developed and used in this study

1 **Table 2 Polymorphic markers developed and used in this study.**

Name	Forward primer (5′-3′)	Reverse primer (5′-3′)
Te20362	TCTACCCTCACCTCGCGTA	TCCATTTCTAATCCACGACCT
Te20811	ATGGAGAAGTAGCAGAGTACATT	TGGATCATCAAAGGCTCACAAA
Te20863	GCCGCCTCTACGAGTT	AAAATCACCCGATTACCACAG
Te20993	AAAATTTAACTTCTGCATGTTG	TCGAGCTTGCATGTCATC
Te21018	GCTTCTAAACTGCTAACAGGT	GTCATTACAATTGCACATAGGAG
Te21052	TCTGAATATTAGCATAGCCGAGT	CTTTTCCTGGGTATATGGAAC
Te21185	ACCGCCGATGACATGC	TCTTCTTCGATCGGGTC

QTL for TGW, GL and GW detected in the ZC9 and Ti52-1 populations

1   **Table 3 QTL for TGW, GL and GW detected in the ZC9 and Ti52-1 populations.**

Population	Segregating region		Trait	QTL	*LOD*	*A*	*D*	*R²* (%)
	Chr	Interval						
ZC9	1	RM12210	GL	*qGL1*	19.1	0.041	-0.007	32.71
			GW	*qGW1*	4.5	-0.007	-0.001	5.84
	9	RM219-RM1896	TGW	*qTGW9*	10.5	0.16	-0.05	19.15
			GW	*qGW9*	5.6	0.007	-0.004	7.42
	10	RM6100-RM1108	GL	*qGL10.1*	6.1	0.020	0.003	9.00
	11	RM224-RM5926	TGW	*qTGW11*	7.8	-0.14	0.023	13.81
			GW	*qGW11*	20.2	-0.016	0.003	31.69
Ti52-1	10	RM25766–RM228	TGW	*qTGW10*	10.1	-0.59	-0.11	24.84
			GL	*qGL10.2*	16.7	-0.081	0.011	36.58
			GW	*qGW10.2*	8.3	-0.024	-0.003	20.15

2   **Notes.**

3   TGW, 1000-grain weight (g); GL, grain length (mm); GW, grain width (mm); Chr, chromosome; *A*, additive effect of

4   replacing a Teqing allele with an IRBB52 allele; *D*: Dominance effect; R^2, proportion of phenotypic variance explained

5   by the QTL effect. QTL were designated following the rule proposed by *McCouch and CGSNL (2008)*.

QTL for TGW, GL and GW detected in five S_1 populations

1   **Table 4 QTL for TGW, GL and GW detected in five S₁ populations.**

Population	Segregating region	Trait	*LOD*	*A*	*D*	*R²* (%)
G10-1	RM25766–Te20811	TGW	n.s.			
		GL	n.s.			
		GW	n.s.			
G10-2	Te20863–Te20993	TGW	14.6	-0.19	0.66	32.09
		GL	28.6	-0.030	0.140	53.10
		GW	8.5	-0.004	0.035	20.12
G10-3	Te20863–RM228	TGW	n.s.			
		GL	n.s.			
		GW	n.s.			
G10-4	Te21185–RM228	TGW	n.s.			
		GL	2.7	0.019	0.023	5.11
		GW	n.s.			
G10-5	RM25845–RM228	TGW	n.s.			
		GL	2.2	0.023	-0.003	3.01
		GW	n.s.			

2   **Notes.**

3   TGW, 1000-grain weight (g); GL, grain length (mm); GW, grain width (mm); *A*, additive effect of replacing a Teqing

4   allele with an IRBB52 allele; *D*, dominance effect; *R²*, proportion of phenotypic variance explained by the QTL effect;

5   n.s., not significant.

QTL for TGW, GL and GW detected in five NIL populations

1   **Table 5 QTL for TGW, GL and GW detected in five NIL populations.**

Name	Segregating region	Trait	Phenotypic mean		P	A	R^2(%)	QTL segregated
			NIL^{TQ}	NIL^{IRBB52}				
G11-1	RM25766–Te20811	TGW	22.37 ± 0.18	22.36 ± 0.25	0.9593			No QTL
		GL	8.953 ± 0.041	8.956 ± 0.044	0.7746			
		GW	2.560 ± 0.015	2.559 ± 0.011	0.6110			
G11-2	Te20863–Te20993	TGW	22.46 ± 0.21	22.03 ± 0.19	<0.0001	-0.21	40.58	*qGS10.1*
		GL	9.009 ± 0.041	8.946 ± 0.038	<0.0001	-0.032	28.55	
		GW	2.568 ± 0.013	2.561 ± 0.012	0.0309	-0.004	3.99	
G11-3	Te20863–RM228	TGW	22.80 ± 0.16	22.06 ± 0.23	<0.0001	-0.37	63.80	*qGS10.1+qGS10.2*
		GL	8.845 ± 0.046	8.767 ± 0.045	<0.0001	-0.039	27.34	
		GW	2.485 ± 0.019	2.446 ± 0.018	<0.0001	-0.020	40.55	
G11-4	Te21185–RM228	TGW	22.89 ± 0.25	22.63 ± 0.41	<0.0001	-0.13	17.59	*qGS10.2*
		GL	8.839 ± 0.058	8.823 ± 0.075	0.4628			
		GW	2.483 ± 0.021	2.460 ± 0.024	<0.0001	-0.012	24.56	
G11-5	RM25845–RM228	TGW	22.04 ± 0.27	21.80 ± 0.28	<0.0001	-0.12	10.32	*qGS10.2*
		GL	8.645 ± 0.054	8.664 ± 0.046	0.0356	0.010	2.29	
		GW	2.480 ± 0.017	2.459 ± 0.017	<0.0001	-0.011	17.51	

2   **Notes.**

3   TGW, 1000-grain weight (g); GL, grain length (mm); GW, grain width (mm); A, additive effect of replacing a Teqing allele with

4   an IRBB52 allele; R^2, proportion of phenotypic variance explained by the QTL effect. NIL^{TQ} and NIL^{IRBB52} are near-isogenic lines

5   with Teqing and IRBB52 homozygous genotypes in the segregating region, respectively.