Quantitative trait loci associated with straighthead-resistance used for marker assisted selection in rice (*Oryza sativa* L.) RIL populations

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Straighthead is a physiological disorder of rice (*Oryza sativa* L.), which causes dramatic yield lose in susceptible cultivars. This disorder was found worldwide, and especially reported to happen increasingly in southern U.S.. Genetically resistant breeding has been considered as the one of most efficient way for straighthead prevention since the traditional prevention method wastes water, and costs labor. In this study, using our previous five markers linked to straighthead Quantitative trait loci (QTLs), AP3858-1 (*qSH-8*), RM225 (*qSH-6*), RM2 (*qSH-7*), RM206 (*qSH-11*) and RM282 (*qSH-3*), we analyzed the genetic effect of the five QTLs on the recombinant inbred lines (RILs) developed from Jing185/Cocodrie and Zhe733/R312 populations. As a result, the RILs having four resistant alleles (at four loci, AP3858-1, RM225, RM2 and RM206) exhibited the highest straighthead resistant. This result suggests that the four markers, especially AP3858-1, are tightly linked to each resistant QTL. Furthermore, Using AP3858-1 we successfully obtained five straighthead-resistant RILs with more than 50% genetic background of Cocodrie. These markers and RILs can be used for future straighthead resistant breeding through marker assistant selection (MAS).

1 **Quantitative trait loci associated with straighthead-resistance used for marker**

2 **assisted selection in rice (*Oryza sativa* L.) RIL populations**

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Abstract

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13 in susceptible cultivars. This disorder was found worldwide, and especially reported to happen

14 increasingly in southern U.S.. Genetically resistant breeding has been considered as the one of

15 most efficient way for straighthead prevention since the traditional prevention method wastes

16 water, and costs labor. In this study, using our previous five markers linked to straighthead

17 Quantitative trait loci (QTLs), AP3858-1 (*qSH-8*), RM225 (*qSH-6*), RM2 (*qSH-7*), RM206 (*qSH-*

18 *11*) and RM282 (*qSH-3*), we analyzed the genetic effect of the five QTLs on the recombinant

19 inbred lines (RILs) developed from Jing185/Cocodrie and Zhe733/R312 populations. As a result,

20 the RILs having four resistant alleles (at four loci, AP3858-1, RM225, RM2 and RM206) exhibited

21 the highest straighthead resistant. This result suggests that the four markers, especially AP3858-1,

22 are tightly linked to each resistant QTL. Furthermore, Using AP3858-1 we successfully obtained

23 five straighthead-resistant RILs with more than 50% genetic background of Cocodrie. These

24 markers and RILs can be used for future straighthead resistant breeding through marker assistant

25 selection (MAS).

26 Key word: straighthead, resistant breeding, QTLs, gene effect, marker assistant selection, *Oryza*
27 *sativa* L.

Introduction

29 Straighthead is a physiological disorder of rice that characterized by sterile florets and distorted

30 spikelets (Yan et al., 2005). It even makes rice kernel empty, panicle erect, and the panicle fail to

31 head out as well. Resultantly, straighthead often causes dramatic yield loss of susceptible cultivars

32 (Dilday et al., 2000). Straighthead was first reported in U.S. (Wells and Gilmour, 1977), and also

33 found in Japan (Takeoka et al., 1990), Australia (Dunn et al., 2006), Portugal (Cunha & Baptista,

34 1958), Thailand (Weerapat, 1979), and Argentina (Yan et al., 2010). It becomes a huge threat to

35 rice production in southern U.S. and worldwide.

36   According to previous studies, straighthead could be caused by many factors, such as sandy to

37 silt loam textured soils [9], low free iron and low pH in soil [10,11], minerals of As, Mn, Ca, and

38 S and soil organic matter [9,12]. In southern U.S., arsenic-based herbicides, e.g. monosodium

39 methanearsonate (MSMA), have been widely applied in the cotton-growing areas. Thus, arsenic

40 (As) usually residues in the paddies. As toxicity exhibits a serial of symptoms in rice, such as

41 decrease in plant height and tillers (Kang et al., 1996), reduction in shoot and root growth

42 (Dasgupta et al., 2004; Rahman et al. 2012), inhibition of seed germination (Shri et al., 2009;

43 Rahman et al., 2012), decline in chlorophyll content, photosynthesis, and sometimes plant death

44 (Rahman et al. 2007). Notably, As could cause typical straighthead symptom for susceptible rice

45 cultivars in MSMA application soil (Rahman et al., 2008; Lomax et al., 2012). Thus, MSMA-

46 induce was a common method on evaluating rice straighthead (Slaton et al., 2000; Wlison et al.,

47 2001).

48   For straighthead prevention, one method is water management called "Draining and Drying".

49 Using this method, farmers need to drain their rice field about 2 weeks after a permanent flood,

50 and then wait for re-flooding until rice leaves exhibit drought stress symptoms (Rasamivelona et

51 al., 1995; Slaton et al., 2000). In Arkansas, one third of the rice field applies D&D method and

52 results in a waste of approximate 150 million m^3 of irrigation water every year (Wilson and

53 Runsick, 2008). Obviously, the method costs nature resources, manpower and also leads to

54 drought-related yield loss.

55   Resistant breeding was considered as the most efficient and environment friendly strategy for

56 straighthead prevention. A number of resistant germplasms have been identified and the genetic

57 base of straighthead has been explored (Yan et al., 2002; Pan et al, 2012). MAS has been used for

58 resistant breeding for many years, and has been demonstrated as a feasible strategy in multiple

59 crops (Yan et al., 2005). In our previous study (Pan et al., 2012), we constructed two recombinant

60 inbred line (RIL) F_9 populations using two resistant parents, Zhe733 and Jing185, and the

61 susceptible parents, Cocodrie and R312. Five QTLs, *qSH-3, qSH-6, qSH-7, qSH-8* and *qSH-11*,

62 were identified to associated with straighthead by linkage mapping using the two RIL populations.

63 Four QTLs (*qSH-6, qSH-7, qSH-8* and *qSH-11*) for Zhe733/R312 population and two QTLs (*qSH-*

64 *3* and *qSH-8*) for Cocodrie/Jing185 population. Among these QTLs, *qSH-8* within 290 kb on

65 chromosome 8 was both identified in two populations. Also, *qSH-8* was confirmed in F_2 and $F_{2:3}$

66 population of Zhe733/R312 (Li et al., 2016b). Therefore, *qSH-8* was proved as a major QTL for

67 straighthead-resistance. Furthermore, five markers, RM282, RM225, RM2, AP3858-1 and RM206

68 (Table S1), were identified to associated with five QTLs, respectively.

69 Arkansas accounts for a large part of rice production of U.S., many cultivars grown in this

70 region are highly susceptible to straighthead. For instance, Cocodire, a major grown cultivar in

71 Arkansas, lost yield up to 94% when straighthead occurred (Linscombe et al., 2000; Wilson et al.,

72 2001). Thus, it is necessary to genetically improve the straighthead-resistance to ensure high yield

73 of rice. In the present study, our objective was to identified the RILs with straighthead-resistant

74 QTLs, and similar agro-traits and background of Cocodrie in Cocodrie/Jing185 population. These

75 RILs could be used for further resistant breeding.

Materials & Methods

Plant material

78 Two RIL F_9 populations, Zhe733/R312 and Cocodire/Jing185, were developed and evaluated

79 straighthead previously (Fig.1) (Pan et al., 2012). Resistant cultivar Zhe733 (PI 629016), Jing185

80 (PI 615205) and susceptible cultivar R312 (PI 614959) were from China, another susceptible

81 Cocodrie (PI 606331) is a widely grown USA cultivar. All three cultivars from China belong to

82 the *indica*, while Cocodrie belong to the *japonica*. A total of 170 F_9 RILs were developed in

83 Zhe733/R312 population while 91 F_9 RILs were produced in Cocodrie/Jing185 population.

Phenotyping

85 Both Zhe733/R312 and Cocodire/Jing185 populations were planted in MSMA-treated soil at

86 Dale Bumpers National Rice Research Center near Stuttgart, Arkansas during two years (2010 and

87 2011). Using a randomized complete block design, RILs of the two F_9 populations were planted

88 in single row field plots (0.62 m^2) with three replications as previously described (Pan et al., 2012).

89 6.7 kg ha^{-1} of MSMA was applied to the soil surface and incorporated prior to planting as

90 previously described (Yan et al., 2005). The four parents, Zhe733, R312, Cocodire, and Jing185,

91 were repeatedly planted in each field tier of 99 rows as controls. Field management was performed

92 as previously described in (Yan et al., 2008).

93   Evaluation of Straighthead rating was based on floret sterility and panicle development using

94   a scale from 1 to 9 at maturity stage (Yan et al., 2005). Score of 1 represented normal plants with

95   panicles fully emerged and more than 80% grains developed, and 9 was sterile plant with no

96   panicle emergence and complete absence of developed grains. Based upon our previous research,

97   RILs with a score of 4.0 or below were resistant, which had 41-60% of seed set or higher than that,

98   while RILs with a score of 6.0 or above were susceptible, which had 11-20% of seed set or lower

99   than that (Li, et al., 2016b).

100   The Cocodrie/Jing185 population was then planted in the clean soil without MSMA involved

101   at Dale Bumpers National Rice Research Center near Stuttgart, Arkansas during two years (2010

102   and 2011). To provide more reliable evaluation, we carried out water management to prevent

103   striaghthead. We conducted a randomized complete block design for field experiments. RILs was

104   planted in single row field plots (0.62 m^2) with three replications in each year. The parents were

105   repeatedly planted in field tier of 99 rows as controls.

106   Evaluation of heading date, height, and tillers were conducted in the field. Heading date was

107   recorded for each plot when 50% the panicles had emerged from the rice culms using visual

108   estimation. Height and tillers were assessed at mature stage using three central individuals of each

109   plot, and plant height was characterized from ground to tip of rice panicle (Counce et al., 2000).

110   The three central individuals of each plot were harvested and air-dried in green house for

111   evaluation of biomass.

112   **Genotyping and genetic Analysis**

113   DNA was extracted from each RIL of two populations and their parents following the CTAB

114   method descried by Hulbert and Bennetzen (1991). The straighthead-linked markers, RM282,

115   RM225, RM2, AP3858-1 and RM206 were used to screen the RILs of the two populations,

116   respectively.

117   DNA amplification was applied as previously described (Pan et al., 2012). As to genotyping,

118   alleles corresponding to resistant or susceptible parents were noted as "a" or "b", respectively.

119   RILs with both alleles were noted as "h". Missing data were noted as ".". According to our previous

120   report, the "a" was resistant allele and the "b" was susceptible at each QTL locus of in

121   ZHE733/R312 population. It is worthy to note in Cocodrie/Jing185 population, "a" was resistant

122   and the "b" was susceptible allele at *qSH-8* locus, whereas the "a" was susceptible allele and "b"

123 was resistant allele at *qSH-3* locus. RILs with striaghthead rating $\leqslant$ 4.0 were selected for further

124 allelic analysis by using a number of markers. These markers including RM225, RM2, RM206,

125 RM282 and AP3858-1, were associated with straighthead-resisitance (Pan et al., 2012), which

126 could be useful for MAS.

Identification of RILs and statistical analysis

128   In Cocodrie/Jing185 population, the RILs having > 50% Cocodrie genetic background were

129 selected for further analysis. The agronomic traits of these selected RILs were analyzed by

130 ANOVA (analysis of variance). Duncan's Multiple Range test was performed between selected

131 RILs and Cocodrie based on the agronomic traits. RILs with different allele combinations were

132 compared with RILs without any resistant alleles (RWARA) of these QTLs, using F-test and T-

133 test. All of these statistical procedures were conducted in SAS software v9.1 (SAS Institute Inc.,

134 Cary, NC).

Results

Gene effect of straighthead-related QTLs

137 Four SSR markers linked to straighthead resistant QTLs, i.e. RM225 (*qSH-6*), RM2 (*qSH-7*),

138 RM206 (*qSH-11*) and AP3858-1 (*qSH-8*), were identified in Zhe733/Jing185 population in

139 previous study (Pan et al., 2012). Of these QTLs, 5 RILs with different genotype were selected to

140 compare with each other based on straighthead rating, when the two parents were set as check

141 (susceptible parent R312 had straighthead rating of 8.8 while resistant parent R312 had

142 straighthead rating of 1.2). As a result (Fig 2A), ZR-64 with susceptible alleles at the four loci, had

143 the highest Straighthead rating (8.7). In contrast, the other four, ZR-238, ZR-132, ZR-14 and ZR-

144 83, having at least one resistant allele, showed lower straighthead ratings than others having none.

145 Especially, ZR-83 having four resistant alleles showed the lowest straighthead rating (1.3).

146   Two SSR linked to straighthead-related QTLs, RM282 (*qSH-3*, susceptible QTL) and

147 AP3858-1 (*qSH-8,* resistant QTL), were identified in Cocodrie/Jing185 population in previous

148 study (Pan et al., 2012). Four RILs were selected to compare with each other based on straighthead

149 rating. The two parents were set as controls (the susceptible parent "Cocodrie" had straighthead

150 rating of 9.3 while the resistant parent "Jing185" had straighthead rating of 2.2). As a result (Fig

151 2B), RIL CJ-405 having no resistant alleles at both loci, showed a very high straighthead rating of

152 9.0. CJ-522 having one resistant allele at RM282, showed straighthead rating of 7.2. CJ-407 having

153 only resistant alleles at AP3858-1, showed straighthead rating of 2.7. Furthermore, CJ-427 having

154 both resistant alleles show straighthead rating of 1.8. Obviously, *qSH-8* was the largest one

155 contributing to resistance. The RILs, CJ-407and CJ-427 with the major resistant QTL could be

156 used as elite lines for further straighthead-resistant breeding program.

157 **Allelic analysis of straighthead related QTLs in Zhe733/R312 and Cocodrie/Jing185**
158 **populations**

159 To investigate effects of five straighthead-related QTLs, 147 RILs from Zhe733/R312 (Table

160 S2) and 91 RILs (Table S3) from Cocodrie/R312 were used in this study. Of Zhe733/R312

161 population (Fig. 3A) (Table 1), 16 RILs without any resistant allele (RWARA-ZR) exhibited mean

162 of straighthead rating 8.66. Six RILs with one resistant allele of *qSH-6* (RM225), exhibited mean

163 of straighthead rating 8.18. Similarly, the RILs with their resistant alleles of *qSH-7* (RM2) and

164 *qSH-11* (RM206) showed mean of straighthead ratings 8.55 and 8.29, respectively. Eight RILs

165 with resistant alleles at the three loci (*qSH-6*qSH-7*qSH-11*) exhibited much lower mean of

166 straighthead rating 3.0. Seven RILs with the resistant allele at *qSH-8* (AP3858-1) showed mean of

167 straighthead rating 5.24. Moreover, the RILs having combination of the resistant allele of *qSH-8*

168 (AP3858-1) with one of any other three loci showed mean of straighthead rating 5.80 (*qSH-

169 11*qSH-8*), 4.88 (*qSH-6*qSH-8*), and 4.45 (*qSH-7*qSH-8*), respectively. The RILs having three

170 resistant alleles showed mean of straighthead rating 2.84 (*qSH-6*qSH-7*qSH-11*), 1.75 (*qSH-

171 6*qSH-7*qSH-8*), 2.11 (*qSH-6*qSH-11*qSH-8*), and 1.95 (*qSH-7*qSH-11*qSH-8*), respectively.

172 The lowest straighthead rating (1.64) was identified in the 5 RILs with the resistant alleles at all

173 of the four loci (*qSH-6*qSH-7*qSH-11*qSH-8*). There were significant differences between all

174 the resistant RILs and RWARA-ZR, while no significant differences between all susceptible RILs

175 and RWARA-ZR (Fig. 3A).

176 In the Cocodrie/Jing185 population (Fig. 3B) (Table 2), 15 RILs had no resistant allele at

177 both loci (RWARA-CJ) exhibited the highest mean of straighthead rating 8.41. Sixteen RILs had

178 one resistant allele *qSH-3* (RM282) with mean of straighthead rating 8.07. Twenty-two RILs had

179 only resistant allele of *qSH-8* (AP3858-1) with mean of straighthead rating of 4.51. Eleven RILs

180 having both resistant alleles of *qSH-3* and *qSH-8* exhibited the lowest mean of straighthead rating

181 3.62. There were significant differences between RILs with the resistant allele of *qSH-8* and with

182   both resistant alleles of *qSH-3* and *qSH-8,* and RWARA-CJ, while no significant differences

183   between RILs with one resistant allele of *qSH-3* and RWARA-CJ (Fig. 3B).

184   **Agronomic analysis in both the RIL populations and straighthead-resistant RILs.**

185   Performed water management, we did not observe striaghthead symptom in both parents and

186   91 RILs of Cocodrie/Jing185 population. This showed that straightheaded was successfully

187   prevented by the water management. The frequency distribution of four traits including heading

188   date, plant height, tillers and biomass, were investigated respectively (Fig. 4). Then ANOVA of

189   the four traits showed that that the four traits were significantly different among RILs in

190   Cocodrie/Jing185 population, respectively (p<0.01).

191   A total of 27 straighthead-resistant RILs with at least resistant allele at AP3858-1 were

192   selected for analysis. Then, 166 polymorphism markers were used to analyze genetical background

193   between the selected RILs and their susceptible parent Cocodrie. As a result, 5 RILs, CJ-404, CJ-

194   407, CJ-479, CJ-480 and CJ-506, shared more than 50% genotypic background of Cocodrie (Table

195   4), and RIL506 showed the highest genetical similarity of 66.0% among these RILs. These RILs

196   and the two parents were subject to phenotypical similarity analysis using Duncan's multiple test

197   (Table 5 and 6). There was significant difference in heading days between Cocodrie and all RILs

198   (Table 6). CJ-479 had the longest heading day among the RILs, while CJ-480 had the shortest one.

199   (Table 5). There was significant difference in plant height between all RILs and Cocodrie, except

200   RIL480 (Table 6). CJ-479 had the highest plant heights while CJ-506 is the shortest one (Table 5).

201   However, there was no significant differences in tillers and biomass between RILs with Cocodrie

202   background (Table 6). Conclusively, all of the five RILs having more than 50% genotypic

203   background of Cocodrie showed high yield similar to Cocodrie's. These RILs are potential

204   germplasm for straighthead-resistant breeding.

205   **Discussion**

206   With the discovery and application of molecular markers in the late 1970s, MAS provided a time-

207   saving and purpose-directing strategy for plant breeding superior to conventional strategy.

208   Previous studies reported MAS application in different species and traits (Chen et al., 2008; Huang

209   et al., 1997; Li et al., 2018; Zhao et al., 2012). According to our previous report (Pan et al., 2012),

210   the straighthead-resistant QTL *qSH-8* accounted for about 67% of phenotypic variation in

211   Cocodrie/Jing185 population, which is much higher than any other QTL. In our present study,

212   AP3858-1 tightly linked to the major *qSH-8* was applied to screen 91 RILs from Cocodrie/Jing185

213   population. As a result, 22 RILs with resistant allele of *qSH8* (AP3858-1) showed mean of

214   striaghthead rating 4.51 (medium resistant). The result suggests that AP3858-1 is a reliable marker

215   for straighthead resistant selection. The three other QTLs in Zhe733/R312 population, *qSH-6*,

216   *qSH-7*, and *qSH-11* explained 13%, 12% and 8% of phenotypic variation, respectively. Although

217   the three QTLs explained much lower variation than *qSH-8*, the QTLs with even low gene effect

218   can be useful when used in other genetic background and also help us understand genetic architect

219   of the interested trait. For instance, A total of 49 QTLs for 14 traits of rice were reported by Wang

220   et al. (2011), eight of them were related to spikelet number per panicle and 1000-grain yield,

221   which explain about 8% and 10% of phenotypic variation, respectively. These QTLs were

222   introduced into the chromosome segment substitution lines (CSSLs) and these lines turned out to

223   have increased panicle and spikelet size as compared with their parent 93-11 (Zong et al., 2012).

224   Based on our study, RILs pyramiding all the three QTLs showed increasing level of straighthead-

225   resistace as compared with susceptible parent R312. This suggests that the three QTLs could be

226   used in marker-assisted selection for resistance.

227   In our study, the QTLs were related to MASA-induced straighthead. In previous studies on

228   As-plant interaction, a number of QTLs were identified to correlated with As tolerance

229   (Ehasanullah & Meetu, 2018; Syed et al., 2016; Xu et al., 2017), and accumulation (Song et al.,

230   2014; Wang et al., 2016; Yamaji & Ma, 2011), respectively. Interestingly, some of these QTLs

231   shared regions with our straighthead-resistant QTLs in rice. For instance, Syed et al. (2016)

232   reported three QTLs, *qAsTSL8, qAsTRL8* and *qAsTRSB8*, which were associated with shooting

233   length, root length and root-shooting biomass under As stress, respectively. Wang et al. (2016)

234   reported a gene *OsPT8* which was related to AsV transportation in the root cell and root-elongation

235   inhibition. Kuramata et al. (2013) reported a gene *qDMAs6.2* which was associated with As

236   accumulation in rice grain. In fact, researchers have already connected striaghthead with As

237   accumulation. Yan et al. (2008) reported that As concentration of straighthead-resistant cultivar

238   Zhe733 was much lower than susceptible cultivar Cocodrie when they were planted in the same

239   soil condition. One also found that As concentration in Cocodrie was nearly 3 times higher than

240   Zhe733 when gown in MASA soil (Hua et al., 2011). Therefore, the QTLs resistant to striaghthead

241   may be also tolerate to As stress. These QTLs will help understand the mechanism underlying As

242   transportation and accumulation in plant.

243   Although breeding for straighthead-resistance has been conducted since 1950s, little progress

244   has been made until 2002 (Yan et al., 2002). One of most important factors was the lack of resistant

245   germplasms in U.S.. Southern U.S. produces over 80% of rice, and 90% cultivars grown here were

246   *tropical japonica* (Mackill & Mckenzie, 2002), whereas most of these cultivars were susceptible

247   to straighthead. In previous studies, 42 resistant accessions were identified from a survey of 1002

248   germplasm collected on a world-wide scale. None of these accessions were *japonica* (Agrama &

249   Yan, 2010), while most of the resistant accessions were classified into *indica* sub-species. Possibly,

250   the straighthead-resistance come from *indica,* and thus the resistance would be used to improve

251   the susceptible cultivars grown in southern U.S.. In fact, the two resistant parents in present study

252   are both from *indica* accessions, but incompatibility between two sub-species was observed.

253   Straighthead evaluation was based on rice infertility, and therefore incompatibility not only made

254   us hard to get the well-developed seeds but also may cause bias when straighthead of the offspring

255   was evaluated. In our previous research for instance (Pan et al., 2012), in some cases, 13 RILs with

256   resistant alleles showed high straighthead rating, which is due to the incompatibility between two-

257   subspecies. In the present study, we identified 5 F_9 RILs from the crossing between *japonica*

258   Cocodrie and *indica* Jing185. These RILs had a major straighthead-resistant QTL *qSH-8*, which is

259   similar to Cocodrie both genotypically and phenotypically. The result suggests that the 5 F_9 RILs,

260   with both *japonica* genetic background and straighthead-resistance, are potential lines to develop

261   *japonica* cultivar for straighthead resistant breeding.

262 **Conclusions**

263   In this study, *qSH-8* was a major QTL for straighthead-resistance, and AP3858-1 linked to *qSH-8*

264   was an ideal tool in marker assistant breeding for straighthead-resistance. In this study, five RILs

265   from Cocodire/Jing185 F_9 population contained resistant alleles of *qSH-8*. Also, they had more

266   than 50% genotypic background of Cocodrie. Compared to Cocodrie, these lines exhibited

267   significant difference in heading date and plant height, but no significant difference in tillers and

268   biomass. What's the most important, these RILs exhibited high yield similar to Cocodire's. The

269   genotypically and phenotypic diverse RILs are potential germplasm which could be used in

270   straighthead-resistant breeding.

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403

Table 1(on next page)

Genetic analysis of straighthead-associated QTLs in MASA-inducedZhe733/R312 population.

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1 **Table 1:**

2 **Genetic analysis of straighthead-associated QTLs in MASA-induced**

3 **Zhe733/R312 population.**

QTLs	Genotype[1]	No. of RILs	Straighthead rating[2]
RWARA-ZR	b*b*b*b	16	8,66±0.35
qSH-6	a	6	8.18±0.65
qSH-7	a	13	8.55±0.35
qSH-11	a	10	8.29±0.45
qSH-8	a	7	5.24±2.83
qSH-6*qSH-7	a*a	5	5.80±3.79
qSH-6*qSH-11	a*a	5	8.10±1.24
qSH-7*qSH-11	a*a	6	8.40±0.83
qSH-6*qSH-8	a*a	9	4.88±2.71
qSH-7*qSH-8	a*a	13	4.45±2.71
qSH-11*qSH-8	a*a	4	5.80±2.48
qSH-6*qSH-7*qSH-11	a*a*a	3	3.02±1.54
qSH-6*qSH-7*qSH-8	a*a*a	7	1.55±0.48
qSH-6*qSH-11*qSH-8	a*a*a	6	2.96±2.28
qSH-7*qSH-11*qSH-8	a*a*a	7	1.95±0.67
qSH-6*qSH-7*qSH-11*qSH-8	a*a*a*a	5	1.64±0.44

4 Abbreviations: RILs, recombinant inbred lines. RWARA-ZR: RILs without any resistant allele in

5 Zhe733/R312 population.

6   [1]Alleles of resistant parent "Zhe733".

7   [2]Straighthead rating using a 1-9 scale. Straighthead rating of 4 or below was resistant and 6 or above was

8   susceptible.

9

Table 2(on next page)

Genetic analysis of straighthead-associated QTLs in MASA-induced Cocodrie/Jing185 population.

Abbreviations: RILs, recombinant inbred lines.

[a]alleles of resistant parent "Jing185"

[b]Straighthead rating using a 1-9 scale. Straighthead rating of 4 or belowwas resistant and 6 or above was susceptible.

1 **Table 2:**

2 **Genetic analysis of straighthead-associated QTLs in MASA-induced Cocodrie/Jing185**

3 **population.**

QTLs	Genotype[1]	No. of RILs	Straighthead rating[2]
RWARA-CJ	b*a	15	8.41±0.53
qSH-3	b	16	8.07±0.64
qSH-8	a	22	4.51±1.73
*qSH-8*qSH-3*	a*b	11	3.62±1.86

4

5 Abbreviations: RILs, recombinant inbred lines. RWARA-CJ: RILs without any resistant allele in

6 Cocodrie/Jing185 population.

7 [1] "a" represents susceptible alleles of parent "Jing185" and "b" represents resistant alleles of parent

8 "Cocodrie" at *qSH-3* locus, meanwhile, "a" represents resistant alleles of parent "Jing185" and "b" represents

9 susceptible alleles of parent "Cocodrie" at *qSH-8* locus.

10 [2]Straighthead rating using a 1-9 scale. Straighthead rating of 4 or below was resistant and 6 or above was

11 susceptible.

12

Table 3(on next page)

ANOVA of four agronomic traits under water-management

Abbreviations: df, differences; Sig, Significant.

**Significantly different from zero at the 0.01 probability level

1 **Table 3：**

2 **One-way ANOVA of four agronomic traits under water-management**

Source		Sum of Squares	Degrees of freedom	Mean Square	F-value	P-value
Heading days	Mean squared between	27210.667	92	295.768	49.148	4.621E-95**
	Mean squared error	1119.333	186	6.018		
	Total	28330	278			
Height	Mean squared between	39906.708	92	433.769	29.036	1.040E-72**
	Mean squared error	2659.144	178	14.939		
	Total	42565.852	270			
Tillers	Mean squared between	15604.872	92	169.618	2.913	6.816E-09**
	Mean squared error	10304.925	177	58.22		
	Total	25909.797	269			
Biomass	Mean squared between	355919.904	92	3868.695	2.743	1.042E-07**
	Mean squared error	231262.864	164	1410.139		
	Total	587182.768	256			

3 **Significantly different from zero at the 0.01 probability level

4

Table 4 (on next page)

Genotypic similarity analysis of RILs of MASA-induced Cocodrie/Jing185 population

[a]"a" represents alleles of resistant parent "Jing185" at *qSH-3* locus, "b" represents susceptible parent "Cocodrie" at *qSH-3* locus.

[b]"a" represents alleles of resistant parent "Jing185" at *qSH-8* locus, "b" represents susceptible parent "Cocodrie" at *qSH-8* locus.

[c]Straighthead rating using a 1-9 scale. was averaged over 3 replications each year and 2 years for which the SD was estimated. Straighthead rating of 4 or below was resistant and 6 or above was susceptible.

1   **Table 4:**

2   **Genotypic similarity analysis of RILs of   MASA-induced Cocodrie/Jing185**

3   **population**

RILs	*qSH-3* genotype[1]	*qSH-8* genotype[2]	Ancestry of Cocodrie	Straighthead rating[3]
CJ-404	a	a	50.64%	3.67±1.63
CJ-407	b	a	53.91%	1.83±0.75
CJ-479	b	a	52.42%	2.67±1.03
CJ-480	b	a	52.40%	3.50±1.83
CJ-506	b	a	66.02%	2.33±1.03
CJ-388	a	a	49.62%	3.00±1.26
CJ-427	a	a	47.73%	2.00±1.26
CJ-478	a	a	44.53%	3.83±1.83

4   [1]"a" represents susceptible alleles of parent "Jing185" while "b" represents resistant alleles of parent

5   "Cocodrie"at *qSH-3* locus.

6   [2]"a" represents resistant alleles of parent "Jing185" and "b" represents susceptible alleles of parent

7   "Cocodrie"at *qSH-8* locus.

8   [3]Straighthead rating using a 1-9 scale. was averaged over 3 replications each year and 2 years for which the

9   SD was estimated. Straighthead rating of 4 or below was resistant and 6 or above was susceptible.

10

Table 5(on next page)

Yield-related characteristics in Cocodrie/Jing185 population under water-management

1　　**Table 5:**

2　　**Yield-related characteristics in Cocodrie/Jing185 population under water-**

3　　**management**

RILs	Heading Date	Plant Height (cm)	Tillers	Biomass (kg)
CJ-404	215.33±0.58	91.44±3.37	22.56±1.83	125.79±17.58
CJ-407	216.67±5.51	88.00±0.94	31.00±3.29	114.24±25.14
CJ-479	236.67±2.89	127.89±2.45	23.89±0.96	155.68±33.06
CJ-480	212.33±1.54	95.78±8.06	25.17±2.59	134.38±10.77
CJ-506	215.00±0.00	65.11±3.01	29.39±8.18	102.73±11.03
Cocodire	223.00±1.00	102.67±2.91	19.89±6.62	147.98±41.26

4

Table 6(on next page)

Duncan's multiple tests of yield-related characteristics in Cocodrie/Jing185 population under water-management

1 **Table 6：**

2 **Duncan's multiple tests of yield-related characteristics in Cocodrie/Jing185 population**

3 **under water-management**

RILs	N	Heading date	Plant height	Tillers	Biomass
		Duncan grouping			
CJ-404	3	B	B	A	A B
CJ-407	3	A B	B	A	A B
CJ-479	3	D	E	A	A B
CJ-480	3	A B	B C	A B	A B
CJ-506	3	B	A	A	A
Cocodrie	3	C	C	A	A B

4

Figure 1

Straighthead phenotype of parents, Zhe733 (resistant)/R312 (susceptible) and Cocodire(susceptible)/Jing185 (resistant).

Figure 1 Straighthead phenotype of parents, Zhe733 (resistant)/R312 (susceptible) and Cocodire(susceptible)/Jing185 (resistant). (Pan et al., 2012)

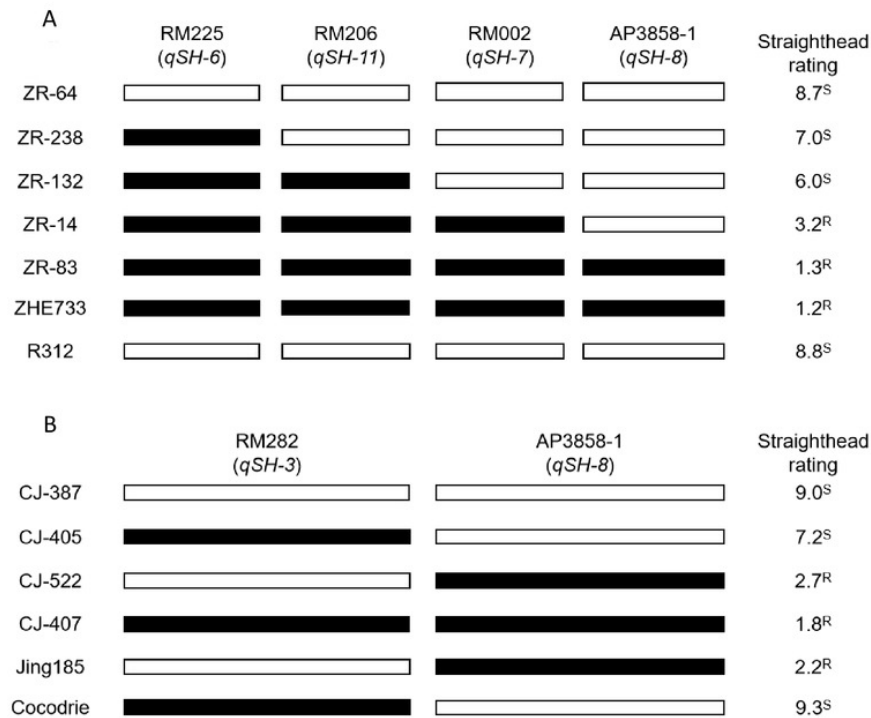# Figure 2

Straighthead rating of RILs with different genotype in Zhe733/R312 population (a) and Cocodrie/Jing185 population (b).

SSR markers RM282, RM225, RM2, AP3858-1 and RM206 were previously identified to

associated with five straighthead resistant QTLs *qSH-3*, *qSH-6*, *qSH-7*, *qSH-8* and *qSH-11*,

respectively. Black bar represents resistant "a" allele, white bar represents susceptible "b"

allele in MASA-induced field. [S]: susceptible. [R]: resistant.

Figure 2: Straighthead rating of RILs with different genotype in Zhe733/R312 population (A) and Cocodrie/Jing185 population (B). SSR markers RM282, RM225, RM2, AP3858-1 and RM206 were previously identified to associated with five straighthead resistant QTLs *qSH-3*, *qSH-6*, *qSH-7*, *qSH-8* and *qSH-11*, respectively. Black bar represents resistant allele, white bar represents susceptible allele in MASA-induced field. [S]: susceptible. [R]: resistant.
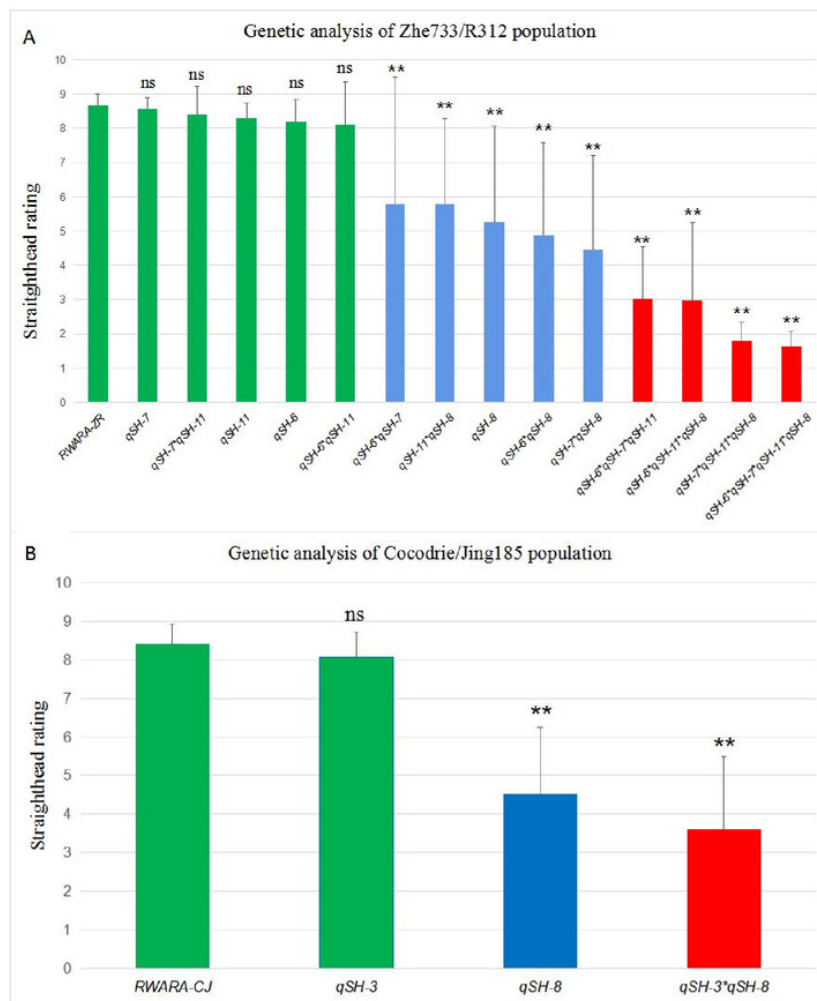
Figure 3

Straighthead rating of RILs with different genotype in MASA-induced Zhe733/R312 population (a) and Cocodrie/Jing185 population (b).

Green bar represents susceptible phenotype with straighthead rating above 6, blue bar represents medium phenotype with straighthead rating between 6 and 4, red bar represents resistant phenotype with straighthead rating blow 4.

Figure 3: Straighthead rating of RILs with different genotype in MASA-induced Zhe733/R312 population (A) and Cocodrie/Jing185 population (B). Green bar represents susceptible phenotype with straighthead rating above 6, blue bar represents medium phenotype with straighthead rating between 6 and 4, red bar represents resistant phenotype with straighthead rating blow 4. RWARA-ZR: RILs without any resistant allele in Zhe733/R312 population. RWARA-CJ: RILs without any resistant allele in Cocodrie/Jing185 population. ns: indicates not significant (p>0.05). **: represents RILs significantly different from RWARA-ZR or RWARA-CJ at the 0.01 probability level in each population, respectively.

Figure 4

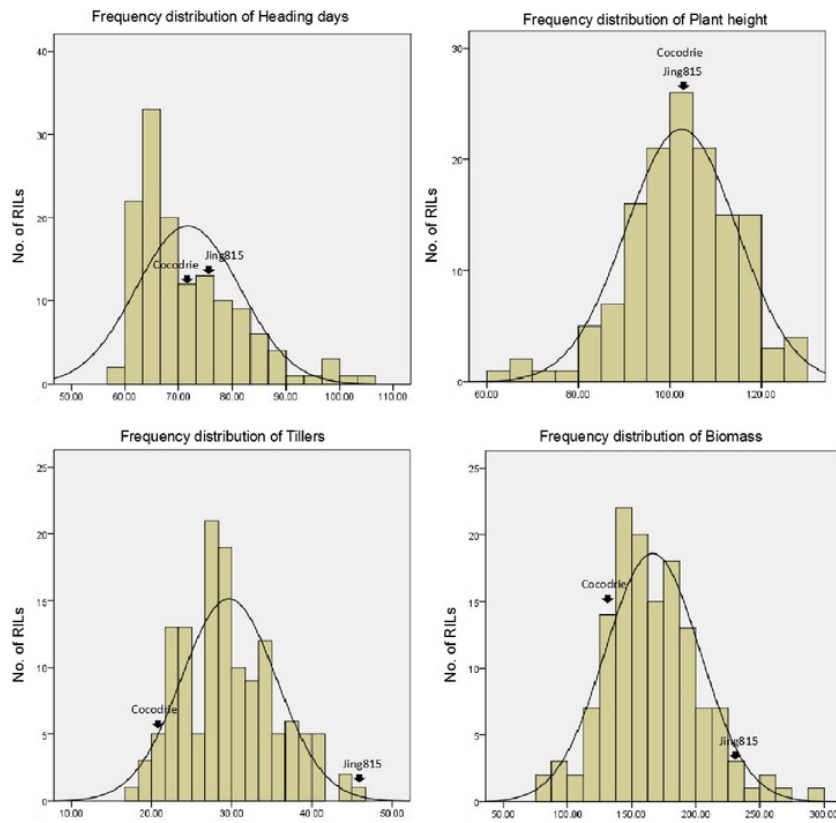Distribution of RILs of Cocodrie/Jing185 population under water-management.

Figure 4: Distribution of RILs of Cocodrie/Jing185 population under water-management.