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).

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

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Abstract

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).

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

Introduction

Straighthead is a physiological disorder of rice that characterized by sterile florets and distorted spikelets (Yan et al., 2005). It even makes rice kernel empty, panicle erect, and the panicle fail to head out as well. Resultantly, straighthead often causes dramatic yield loss of susceptible cultivars (Dilday et al., 2000). Straighthead was first reported in U.S. (Wells and Gilmour, 1977), and also

found in Japan (Takeoka et al., 1990), Australia (Dunn et al., 2006), Portugal (Cunha & Baptista, 1958), Thailand (Weerapat, 1979), and Argentina (Yan et al., 2010). It becomes a huge threat to rice production in southern U.S. and worldwide.

According to previous studies, straighthead could be caused by many factors, such as sandy to silt loam textured soils [9], low free iron and low pH in soil [10,11], minerals of As, Mn, Ca, and S and soil organic matter [9,12]. In southern U.S., arsenic-based herbicides, e.g. monosodium methanearsonate (MSMA), have been widely applied in the cotton-growing areas. Thus, arsenic (As) usually residues in the paddies. As toxicity exhibits a serial of symptoms in rice, such as decrease in plant height and tillers (Kang et al., 1996), reduction in shoot and root growth (Dasgupta et al., 2004; Rahman et al. 2012), inhibition of seed germination (Shri et al., 2009; Rahman et al., 2012), decline in chlorophyll content, photosynthesis, and sometimes plant death (Rahman et al. 2007). Notably, As could cause typical straighthead symptom for susceptible rice cultivars in MSMA application soil (Rahman et al., 2008; Lomax et al., 2012) Thus, MSMA-induce was a common method on evaluating rice straighthead (Slaton et al., 2000; Wlison et al., 2001).

For straighthead prevention, one method is water management called “Draining and Drying”. Using this method, farmers need to drain their rice field about 2 weeks after a permanent flood, and then wait for re-flooding until rice leaves exhibit drought stress symptoms (Rasamivelona et al., 1995; Slaton et al., 2000). In Arkansas, one third of the rice field applies D&D method and results in a waste of approximate 150 million m^3 of irrigation water every year (Wilson and Runsick, 2008). Obviously, the method costs nature resources, manpower and also leads to drought-related yield loss.

Resistant breeding was considered as the most efficient and environment friendly strategy for straighthead prevention. A number of resistant germplasms have been identified and the genetic base of straighthead has been explored (Yan et al., 2002; Pan et al, 2012). MAS has been used for resistant breeding for many years, and has been demonstrated as a feasible strategy in multiple crops (Yan et al., 2005). In our previous study (Pan et al., 2012), we constructed two recombinant inbred line (RIL) F_9 populations using two resistant parents, Zhe733 and Jing185, and the susceptible parents, Cocodrie and R312. Five QTLs, *qSH-3, qSH-6, qSH-7, qSH-8* and *qSH-11*, were identified to associated with straighthead by linkage mapping using the two RIL populations. Four QTLs (*qSH-6, qSH-7, qSH-8* and *qSH-11*) for Zhe733/R312 population and two QTLs (*qSH-*

3 and *qSH-8*) for Cocodrie/Jing185 population. Among these QTLs, *qSH-8* within 290 kb on chromosome 8 was both identified in two populations. Also, *qSH-8* was confirmed in F_2 and $F_{2:3}$ population of Zhe733/R312 (Li et al., 2016b). Therefore, *qSH-8* was proved as a major QTL for straighthead-resistance. Furthermore, five markers, RM282, RM225, RM2, AP3858-1 and RM206 (Table S1), were identified to associated with five QTLs, respectively.

Arkansas accounts for a large part of rice production of U.S., many cultivars grown in this region are highly susceptible to straighthead. For instance, Cocodire, a major grown cultivar in Arkansas, lost yield up to 94% when straighthead occurred (Linscombe et al., 2000; Wilson et al., 2001). Thus, it is necessary to genetically improve the straighthead-resistance to ensure high yield of rice. In the present study, our objective was to identified the RILs with straighthead-resistant QTLs, and similar agro-traits and background of Cocodrie in Cocodrie/Jing185 population. These RILs could be used for further resistant breeding.

Materials & Methods

Plant material

Two RIL F_9 populations, Zhe733/R312 and Cocodire/Jing185, were developed and evaluated straighthead previously (Fig.1) (Pan et al., 2012). Resistant cultivar Zhe733 (PI 629016), Jing185 (PI 615205) and susceptible cultivar R312 (PI 614959) were from China, another susceptible Cocodrie (PI 606331) is a widely grown USA cultivar. All three cultivars from China belong to the *indica*, while Cocodrie belong to the *japonica*. A total of 170 F_9 RILs were developed in Zhe733/R312 population while 91 F_9 RILs were produced in Cocodrie/Jing185 population.

Phenotyping

Both Zhe733/R312 and Cocodire/Jing185 populations were planted in MSMA-treated soil at Dale Bumpers National Rice Research Center near Stuttgart, Arkansas during two years (2010 and 2011). Using a randomized complete block design, RILs of the two F_9 populations were planted in single row field plots (0.62 m^2) with three replications as previously described (Pan et al., 2012). 6.7 kg ha^{-1} of MSMA was applied to the soil surface and incorporated prior to planting as previously described (Yan et al., 2005). The four parents, Zhe733, R312, Cocodire, and Jing185, were repeatedly planted in each field tier of 99 rows as controls. Field management was performed as previously described in (Yan et al., 2008).

Evaluation of Straighthead rating was based on floret sterility and panicle development using a scale from 1 to 9 at maturity stage (Yan et al., 2005). Score of 1 represented normal plants with panicles fully emerged and more than 80% grains developed, and 9 was sterile plant with no panicle emergence and complete absence of developed grains. Based upon our previous research, RILs with a score of 4.0 or below were resistant, which had 41-60% of seed set or higher than that, while RILs with a score of 6.0 or above were susceptible, which had 11-20% of seed set or lower than that (Li, et al., 2016b).

The Cocodrie/Jing185 population was then planted in the clean soil without MSMA involved at Dale Bumpers National Rice Research Center near Stuttgart, Arkansas during two years (2010 and 2011). To provide more reliable evaluation, we carried out water management to prevent striaghthead. We conducted a randomized complete block design for field experiments. RILs was planted in single row field plots (0.62 m^2) with three replications in each year. The parents were repeatedly planted in field tier of 99 rows as controls.

Evaluation of heading date, height, and tillers were conducted in the field. Heading date was recorded for each plot when 50% the panicles had emerged from the rice culms using visual estimation. Height and tillers were assessed at mature stage using three central individuals of each plot, and plant height was characterized from ground to tip of rice panicle (Counce et al., 2000). The three central individuals of each plot were harvested and air-dried in green house for evaluation of biomass.

Genotyping and genetic Analysis

DNA was extracted from each RIL of two populations and their parents following the CTAB method descried by Hulbert and Bennetzen (1991). The straighthead-linked markers, RM282, RM225, RM2, AP3858-1 and RM206 were used to screen the RILs of the two populations, respectively.

DNA amplification was applied as previously described (Pan et al., 2012). As to genotyping, alleles corresponding to resistant or susceptible parents were noted as “a” or “b”, respectively. RILs with both alleles were noted as “h”. Missing data were noted as “.”. According to our previous report, the “a” was resistant allele and the “b” was susceptible at each QTL locus of in ZHE733/R312 population. It is worthy to note in Cocodrie/Jing185 population, “a” was resistant and the “b” was susceptible allele at *qSH-8* locus, whereas the “a” was susceptible allele and “b”

was resistant allele at *qSH-3* locus. RILs with striaghthead rating ⩽ 4.0 were selected for further allelic analysis by using a number of markers. These markers including RM225, RM2, RM206, RM282 and AP3858-1, were associated with straighthead-resisitance (Pan et al., 2012), which could be useful for MAS.

Identification of RILs and statistical analysis

In Cocodrie/Jing185 population, the RILs having > 50% Cocodrie genetic background were selected for further analysis. The agronomic traits of these selected RILs were analyzed by ANOVA (analysis of variance). Duncan's Multiple Range test was performed between selected RILs and Cocodrie based on the agronomic traits. RILs with different allele combinations were compared with RILs without any resistant alleles (RWARA) of these QTLs, using F-test and T-test. All of these statistical procedures were conducted in SAS software v9.1 (SAS Institute Inc., Cary, NC).

Results

Gene effect of straighthead-related QTLs

Four SSR markers linked to straighthead resistant QTLs, i.e. RM225 (*qSH-6*), RM2 (*qSH-7*), RM206 (*qSH-11*) and AP3858-1 (*qSH-8*), were identified in Zhe733/Jing185 population in previous study (Pan et al., 2012). Of these QTLs, 5 RILs with different genotype were selected to compare with each other based on straighthead rating, when the two parents were set as check (susceptible parent R312 had straighthead rating of 8.8 while resistant parent R312 had straighthead rating of 1.2). As a result (Fig 2A), ZR-64 with susceptible alleles at the four loci, had the highest Straighthead rating (8.7). In contrast, the other four, ZR-238, ZR-132, ZR-14 and ZR-83, having at least one resistant allele, showed lower straighthead ratings than others having none. Especially, ZR-83 having four resistant alleles showed the lowest straighthead rating (1.3).

Two SSR linked to straighthead-related QTLs, RM282 (*qSH-3*, susceptible QTL) and AP3858-1 (*qSH-8,* resistant QTL), were identified in Cocodrie/Jing185 population in previous study (Pan et al., 2012). Four RILs were selected to compare with each other based on straighthead rating. The two parents were set as controls (the susceptible parent “Cocodrie” had straighthead rating of 9.3 while the resistant parent “Jing185” had straighthead rating of 2.2). As a result (Fig

2B), RIL CJ-405 having no resistant alleles at both loci, showed a very high straighthead rating of 9.0. CJ-522 having one resistant allele at RM282, showed straighthead rating of 7.2. CJ-407 having only resistant alleles at AP3858-1, showed straighthead rating of 2.7. Furthermore, CJ-427 having both resistant alleles show straighthead rating of 1.8. Obviously, *qSH-8* was the largest one contributing to resistance. The RILs, CJ-407and CJ-427 with the major resistant QTL could be used as elite lines for further straighthead-resistant breeding program.

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

To investigate effects of five straighthead-related QTLs, 147 RILs from Zhe733/R312 (Table S2) and 91 RILs (Table S3) from Cocodrie/R312 were used in this study. Of Zhe733/R312 population (Fig. 3A) (Table 1), 16 RILs without any resistant allele (RWARA-ZR) exhibited mean of straighthead rating 8.66. Six RILs with one resistant allele of *qSH-6* (RM225), exhibited mean of straighthead rating 8.18. Similarly, the RILs with their resistant alleles of *qSH-7* (RM2) and *qSH-11* (RM206) showed mean of straighthead ratings 8.55 and 8.29, respectively. Eight RILs with resistant alleles at the three loci (*qSH-6*qSH-7*qSH-11*) exhibited much lower mean of straighthead rating 3.0. Seven RILs with the resistant allele at *qSH-8* (AP3858-1) showed mean of straighthead rating 5.24. Moreover, the RILs having combination of the resistant allele of *qSH-8* (AP3858-1) with one of any other three loci showed mean of straighthead rating 5.80 (*qSH-11*qSH-8*), 4.88 (*qSH-6*qSH-8*), and 4.45 (*qSH-7*qSH-8*), respectively. The RILs having three resistant alleles showed mean of straighthead rating 2.84 (*qSH-6*qSH-7*qSH-11*), 1.75 (*qSH-6*qSH-7*qSH-8*), 2.11 (*qSH-6*qSH-11*qSH-8*), and 1.95 (*qSH-7*qSH-11*qSH-8*), respectively. The lowest straighthead rating (1.64) was identified in the 5 RILs with the resistant alleles at all of the four loci (*qSH-6*qSH-7*qSH-11*qSH-8*). There were significant differences between all the resistant RILs and RWARA-ZR, while no significant differences between all susceptible RILs and RWARA-ZR (Fig. 3A).

In the Cocodrie/Jing185 population (Fig. 3B) (Table 2), 15 RILs had no resistant allele at both loci (RWARA-CJ) exhibited the highest mean of straighthead rating 8.41. Sixteen RILs had one resistant allele *qSH-3* (RM282) with mean of straighthead rating 8.07. Twenty-two RILs had only resistant allele of *qSH-8* (AP3858-1) with mean of straighthead rating of 4.51. Eleven RILs having both resistant alleles of *qSH-3* and *qSH-8* exhibited the lowest mean of straighthead rating 3.62. There were significant differences between RILs with the resistant allele of *qSH-8* and with

both resistant alleles of *qSH-3* and *qSH-8,* and RWARA-CJ, while no significant differences between RILs with one resistant allele of *qSH-3* and RWARA-CJ (Fig. 3B).

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

Performed water management, we did not observe striaghthead symptom in both parents and 91 RILs of Cocodrie/Jing185 population. This showed that straightheaded was successfully prevented by the water management. The frequency distribution of four traits including heading date, plant height, tillers and biomass, were investigated respectively (Fig. 4). Then ANOVA of the four traits showed that that the four traits were significantly different among RILs in Cocodrie/Jing185 population, respectively ($p<0.01$).

A total of 27 straighthead-resistant RILs with at least resistant allele at AP3858-1 were selected for analysis. Then, 166 polymorphism markers were used to analyze genetical background between the selected RILs and their susceptible parent Cocodrie. As a result, 5 RILs, CJ-404, CJ-407, CJ-479, CJ-480 and CJ-506, shared more than 50% genotypic background of Cocodrie (Table 4), and RIL506 showed the highest genetical similarity of 66.0% among these RILs. These RILs and the two parents were subject to phenotypical similarity analysis using Duncan's multiple test (Table 5 and 6). There was significant difference in heading days between Cocodrie and all RILs (Table 6). CJ-479 had the longest heading day among the RILs, while CJ-480 had the shortest one. (Table 5). There was significant difference in plant height between all RILs and Cocodrie, except RIL480 (Table 6). CJ-479 had the highest plant heights while CJ-506 is the shortest one (Table 5). However, there was no significant differences in tillers and biomass between RILs with Cocodrie background (Table 6). Conclusively, all of the five RILs having more than 50% genotypic background of Cocodrie showed high yield similar to Cocodrie's. These RILs are potential germplasm for straighthead-resistant breeding.

Discussion

With the discovery and application of molecular markers in the late 1970s, MAS provided a time-saving and purpose-directing strategy for plant breeding superior to conventional strategy. Previous studies reported MAS application in different species and traits (Chen et al., 2008; Huang et al., 1997; Li et al., 2018; Zhao et al., 2012). According to our previous report (Pan et al., 2012), the straighthead-resistant QTL *qSH-8* accounted for about 67% of phenotypic variation in Cocodrie/Jing185 population, which is much higher than any other QTL. In our present study,

AP3858-1 tightly linked to the major *qSH-8* was applied to screen 91 RILs from Cocodrie/Jing185 population. As a result, 22 RILs with resistant allele of *qSH8* (AP3858-1) showed mean of striaghthead rating 4.51 (medium resistant). The result suggests that AP3858-1 is a reliable marker for straighthead resistant selection. The three other QTLs in Zhe733/R312 population, *qSH-6*, *qSH-7*, and *qSH-11* explained 13%, 12% and 8% of phenotypic variation, respectively. Although the three QTLs explained much lower variation than *qSH-8*, the QTLs with even low gene effect can be useful when used in other genetic background and also help us understand genetic architect of the interested trait. For instance, A total of 49 QTLs for 14 traits of rice were reported by Wang et al. (2011), eight of them were related to spikelet number per panicle and 1000-grain yield, which explain about 8% and 10% of phenotypic variation, respectively. These QTLs were introduced into the chromosome segment substitution lines (CSSLs) and these lines turned out to have increased panicle and spikelet size as compared with their parent 93-11 (Zong et al., 2012). Based on our study, RILs pyramiding all the three QTLs showed increasing level of straighthead-resistace as compared with susceptible parent R312. This suggests that the three QTLs could be used in marker-assisted selection for resistance.

In our study, the QTLs were related to MASA-induced straighthead. In previous studies on As-plant interaction, a number of QTLs were identified to correlated with As tolerance (Ehasanullah & Meetu, 2018; Syed et al., 2016; Xu et al., 2017), and accumulation (Song et al., 2014; Wang et al., 2016; Yamaji & Ma, 2011), respectively. Interestingly, some of these QTLs shared regions with our straighthead-resistant QTLs in rice. For instance, Syed et al. (2016) reported three QTLs, *qAsTSL8, qAsTRL8* and *qAsTRSB8*, which were associated with shooting length, root length and root-shooting biomass under As stress, respectively. Wang et al. (2016) reported a gene *OsPT8* which was related to AsV transportation in the root cell and root-elongation inhibition. Kuramata et al. (2013) reported a gene *qDMAs6.2* which was associated with As accumulation in rice grain. In fact, researchers have already connected striaghthead with As accumulation. Yan et al. (2008) reported that As concentration of straighthead-resistant cultivar Zhe733 was much lower than susceptible cultivar Cocodrie when they were planted in the same soil condition. One also found that As concentration in Cocodrie was nearly 3 times higher than Zhe733 when gown in MASA soil (Hua et al., 2011). Therefore, the QTLs resistant to striaghthead may be also tolerate to As stress. These QTLs will help understand the mechanism underlying As transportation and accumulation in plant.

Although breeding for straighthead-resistance has been conducted since 1950s, little progress has been made until 2002 (Yan et al., 2002). One of most important factors was the lack of resistant germplasms in U.S.. Southern U.S. produces over 80% of rice, and 90% cultivars grown here were *tropical japonica* (Mackill & Mckenzie, 2002), whereas most of these cultivars were susceptible to straighthead. In previous studies, 42 resistant accessions were identified from a survey of 1002 germplasm collected on a world-wide scale. None of these accessions were *japonica* (Agrama & Yan, 2010), while most of the resistant accessions were classified into *indica* sub-species. Possibly, the straighthead-resistance come from *indica,* and thus the resistance would be used to improve the susceptible cultivars grown in southern U.S.. In fact, the two resistant parents in present study are both from *indica* accessions, but incompatibility between two sub-species was observed. Straighthead evaluation was based on rice infertility, and therefore incompatibility not only made us hard to get the well-developed seeds but also may cause bias when straighthead of the offspring was evaluated. In our previous research for instance (Pan et al., 2012), in some cases, 13 RILs with resistant alleles showed high straighthead rating, which is due to the incompatibility between two-subspecies. In the present study, we identified 5 F_9 RILs from the crossing between *japonica* Cocodrie and *indica* Jing185. These RILs had a major straighthead-resistant QTL *qSH-8*, which is similar to Cocodrie both genotypically and phenotypically. The result suggests that the 5 F_9 RILs, with both *japonica* genetic background and straighthead-resistance, are potential lines to develop *japonica* cultivar for straighthead resistant breeding.

Conclusions

In this study, *qSH-8* was a major QTL for straighthead-resistance, and AP3858-1 linked to *qSH-8* was an ideal tool in marker assistant breeding for straighthead-resistance. In this study, five RILs from Cocodire/Jing185 F_9 population contained resistant alleles of *qSH-8*. Also, they had more than 50% genotypic background of Cocodrie. Compared to Cocodrie, these lines exhibited significant difference in heading date and plant height, but no significant difference in tillers and biomass. What's the most important, these RILs exhibited high yield similar to Cocodire's. The genotypically and phenotypic diverse RILs are potential germplasm which could be used in straighthead-resistant breeding.

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Table 1(on next page)

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

Table 1:

Genetic analysis of straighthead-associated QTLs in MASA-induced 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

Abbreviations: RILs, recombinant inbred lines. RWARA-ZR: RILs without any resistant allele in Zhe733/R312 population.

[1]Alleles of resistant parent “Zhe733”.

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

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.

Table 2:

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

Abbreviations: RILs, recombinant inbred lines. RWARA-CJ: RILs without any resistant allele in Cocodrie/Jing185 population.

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

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

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

Table 3：

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			

**Significantly different from zero at the 0.01 probability level

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.

Table 4:

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

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

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

[3]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.

Table 5(on next page)

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

Table 5:

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

Table 6(on next page)

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

Table 6：

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

RILs	N	Duncan grouping			
		Heading date	Plant height	Tillers	Biomass
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

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.

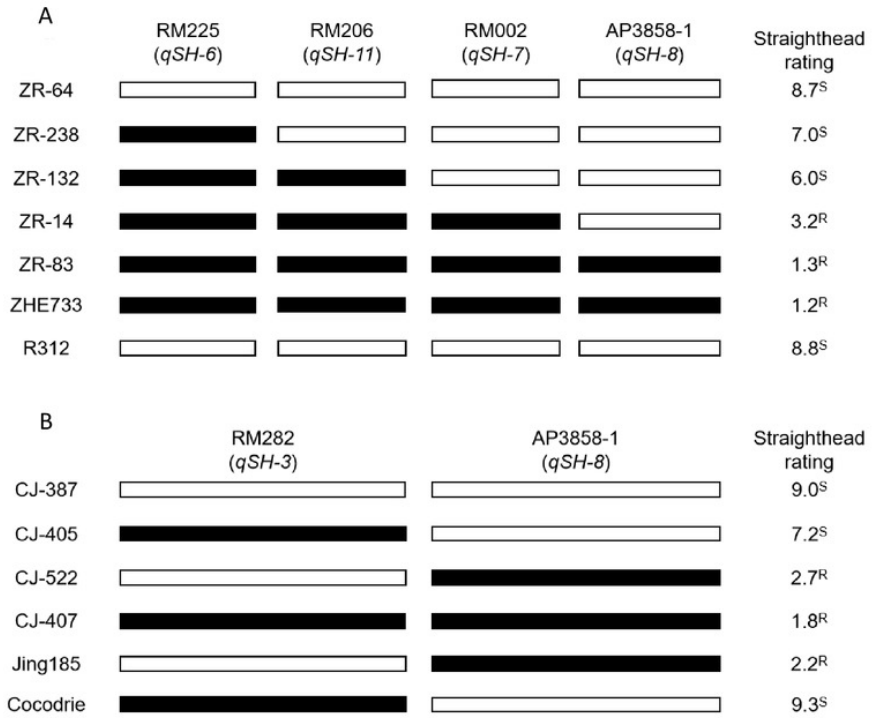

Figure 2: Straighthead rating of RILs with different genotype in Zhe733/R312 population (A) and Cocodrie/Jing185 population (B). SSR markers RM282, RM225, RM2, AP3858-1 and RM206 were previously identified to associated with five straighthead resistant QTLs *qSH-3*, *qSH-6*, *qSH-7*, *qSH-8* and *qSH-11,* 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.

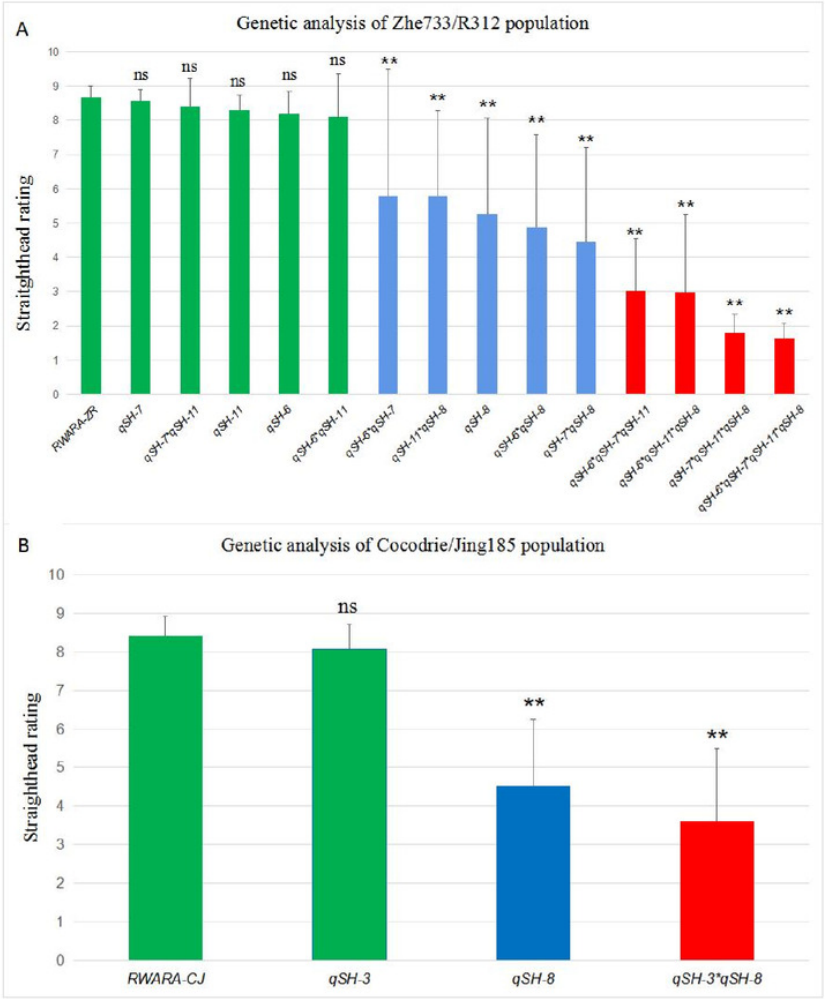

Figure 3: Straighthead rating of RILs with different genotype in MASA-induced Zhe733/R312 population (A) and Cocodrie/Jing185 population (B). Green bar represents susceptible phenotype with straighthead rating above 6, blue bar represents medium phenotype with straighthead rating between 6 and 4, red 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.

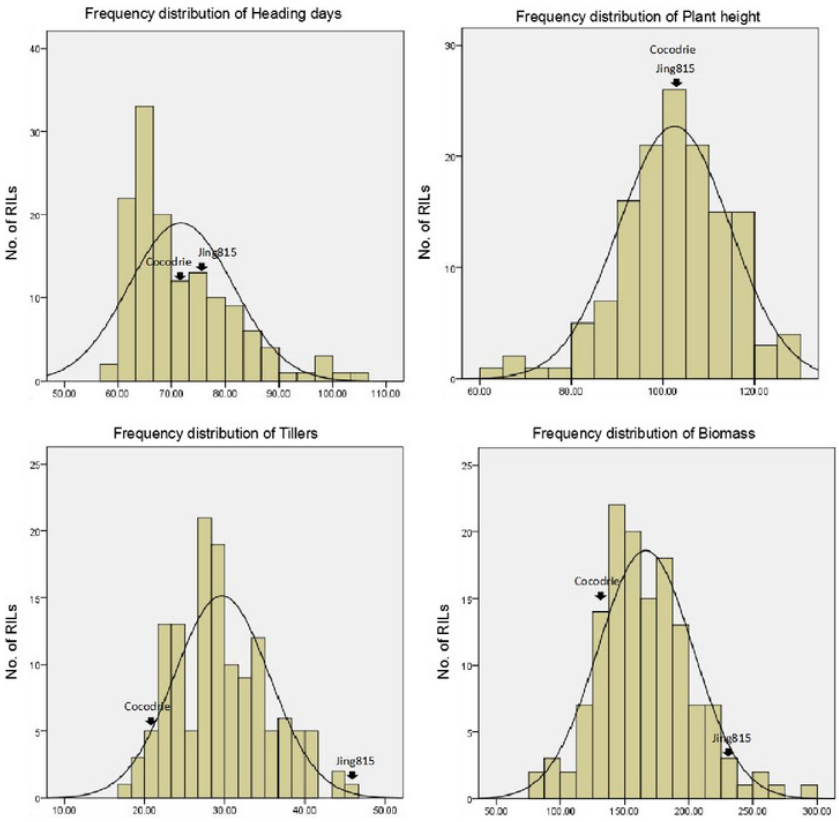

Figure 4: Distribution of RILs of Cocodrie/Jing185 population under water-management.