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ORIGINAL PAPER

Marker-assisted selection and evaluation of the QTL for stigma exsertion under japonica rice genetic background

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Abstract Stigma exsertion is one of the important traits which contribute to the efficient improvement of commercial seed production in hybrid rice. In order to understand the genetic factors involved in the stigma exsertion of an indica variety—IR24—a QTL analysis was conducted using the F_2 population between a japonica variety—Koshihikari—and a breeding line showing exserted stigma selected from the backcross population between IR24 as a donor and japonica varieties. As a result, a highly significant QTL (*qES3*), which had been predicted in the recombinant inbred population of IR24, was confirmed at the centromeric region on chromosome 3. *qES3* increases about 20% of the frequency of the exserted stigmas at the IR24 allele and explains about 32% of the total phenotypic variance. A QTL near-isogenic line for *qES3* increased the frequency of the exserted stigma by 36% compared to that of Koshihikari in a field evaluation, which suggests that *qES3* is a promising QTL for the development of a maternal line for hybrid rice.

Introduction

Breeding of high-yielding hybrid rice is one of the hopeful solutions toward a food shortage problem that is caused by a marked increase in the global population. However, as opposed to the case of open-pollinated plants such as maize or many vegetable plants in which the hybrid system is common, it is difficult to reliably produce an acceptable quantity of hybrid rice seeds, as rice is strictly a self-pollinating plant (Azzini and Rutger 1982). Therefore, improvement of the hybrid seed production efficiency is an essential factor for the commercialization of hybrid rice. There are several phenotypic traits contributing to the hybrid seed production efficiency, for example, flowering behavior (days to heading or blooming time), number of pollens, pollen longevity and morphological traits in a floret such as size of the stigma and style, stigma exsertion or spikelet-opening angle (Virmani 1994). Among them, stigma exsertion is especially emphasized as a component increasing the opportunity of pollination (Kato and Namai 1987b).

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Development of a maternal parent with highly exserted stigmas is expected not only to help to trap more pollen dispersed from a paternal parent, but also to overcome the barrier of pollination caused by the differences in the flowering date or time between the parents.

Since continuous phenotypic variation is broadly observed, stigma exsertion in rice species is thought to be controlled by polygenes (Virmani and Athwal 1973, 1974). Recent progress in the DNA marker technique has been providing genetic information about stigma exsertion applicable to actual breeding. For example, nine QTLs for the frequency of stigma exsertion were detected in the recombinant inbred lines (RILs) derived from the cross between a japonica variety, Asominori, and an indica variety—IR24 (Yamamoto et al. 2003)—and two QTLs for the rate of exserted stigma in the RILs derived from the cross between an indica variety, Pei-Kuh, and a wild rice—W1944 (*Oryza rufipogon* Griff.) (Uga et al. 2003). However, it is still uncertain whether these QTLs effectively work in the genetic background of current candidates of the maternal parent in hybrid rice.

To remove such a concern, Tanksley and Nelson (1996) proposed a strategy of an advanced backcross QTL (AB-QTL), in which valuable QTLs are introgressed with the same timing as the QTL detection during the process of backcrossing using an elite cultivar as the recurrent parent. This method was primarily applied to the tomato (Tanksley et al. 1996) and then applied to rice (Moncada et al. 2001; Thomson et al. 2003; Xiao et al. 1998). In present study, we followed the concept of the AB-QTL strategy to efficiently improve the stigma exsertion in elite japonica cultivar for hybrid seed production. A QTL analysis was conducted in the F_2 population between Koshihikari, the leading variety in Japan, and an intermediate line possessing a character of highly exserted stigmas which were introduced from IR24 under japonica genetic backgrounds. As a result, the prominent QTL was locally detected corresponding to one of the QTLs that had been detected in our previous study where the same donor parent was used (Yamamoto et al. 2003). Also, we examined several agronomic characters in the QTL near-isogenic line (QTL-NIL) for the QTL. The phenotypic effects and the implication of the QTL for practical use in rice breeding are discussed.

Materials and methods

Pedigree chart of the parental line, 98SQ1496

The pedigree chart of 98SQ1496—an intermediate line for stigma exsertion—is shown in Fig. 1 IL223 is an F_5 line possessing a highly exserted stigma trait from IR24 under the genetic background of Hoshinohikari, a japonica variety. In order to replace the genetic background of Hoshinohikari in IL223 with that of Koshihikari, the phenotypic selection of a highly stigma exsertion was carried out among the BC_1F_4 lines derived from the cross of IL223/Koshihikari//Koshihikari. As a result, we developed a desirable intermediate line called 98SQ1496.

Traits evaluation

One hundred and fifty F_2 plants derived from the cross between Koshihikari and 98SQ1496 were grown in a greenhouse during the cropping season in 2000. The germinated seeds were sown on May 17, and the seedlings were transplanted into pots on June 5. The important agricultural traits that are related to the efficiency of the F_1 seed production and/or the grain quality of the hybrid rice, including stigma exsertion, days to heading, panicle length and grain size, were evaluated. At 7–10 days after heading, the length of the longest panicle was measured in each plant. Simultaneously, two normal panicles from each plant were sampled for counting the flowered glumes and exserted stigmas. The frequency of the exserted stigma was defined as the rate (%) of the number of exserted stigmas to the total number of stigmas on the flowered glumes, which is equivalent to the double number of flowered glumes. At maturity, ten matured grains on the primary rachis-branch of the panicle were hulled and used for the measurements of their length and width.

DNA marker analysis

The total DNA was extracted from the green leaves according to the method of Guillemaut and Marechal-Drouard (1992) with minor modification of the extraction buffer: 100 mM Tris pH 8.0, 50 mM EDTA, 500 mM NaCl, 1.4% SDS, and used as a template for the PCR analysis. A total of 269 PCR-based markers were used in this study. They consisted of the markers described in previous reports (Chen et al. 1997; Komori and Nitta 2003, 2005; Komori et al. 2003; Miyao et al. 1996; Panaud et al. 1996; Temnykh et al. 2000; Wu and Tanksley 1993), the markers on the website of the Rice Genome Project in Japan (http://www.rgp.dna.affrc.go.jp) and the markers developed by a sequence comparison between Asominori and IR24 based on previous reports (Chen et al. 1999; Harushima et al. 1998; Mochizuki et al. 1992; Takakura et al. 2000; Tenzen et al. 1994; Ueki et al. 1995; Wu et al. 2002; Xie and Wu 1988; listed in S1). Polymorphism detection in each marker followed the original protocols.

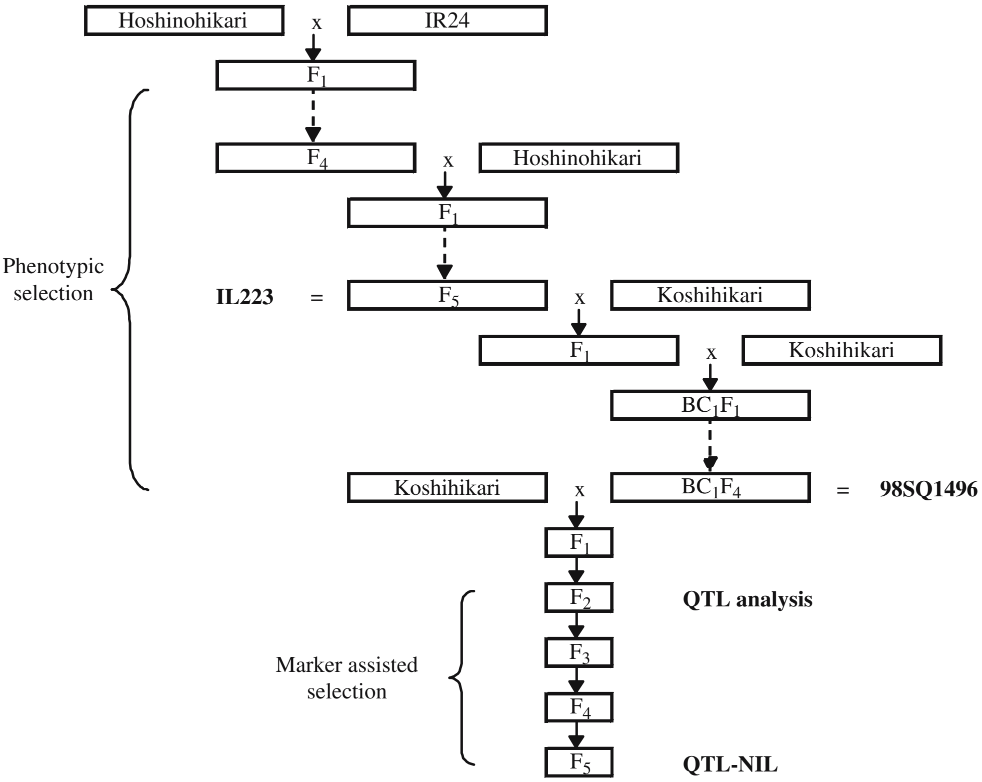


Fig. 1 Pedigree chart of the experimental materials

QTL analysis

The linkage group and orders of the markers in the F_2 population were determined using MAPMAKER/EXP version2.0 (Lander et al. 1987). Consequently, the chromosome number was assigned by referring the RFLP framework map, constructed by using the 71 recombinant inbred lines (RILs) derived from the cross between Asominori and IR24 (Tsunematsu et al. 1996). This map, consisting of 38 markers, covered 229.9 cM of IR24 chromosomal region in 98SQ1496 with an average interval distance of 6.1 cM.

A QTL analysis was performed by composite interval mapping using QTL Cartographer ver. 2.5 (Wang et al. 2006). The putative QTL for each trait was estimated with a calculated LOD score after 1,000 permutation tests.

Selection of the QTL-NIL

The candidate plants for the QTL-NIL were selected based on the genotype data of the F_2 individuals. These plants were homozygous for the IR24 allele in the target region and for the japonica allele in most of the other regions. Twenty-four self-pollinated seeds from the selected F_2 plants were grown in the greenhouse and subjected to marker-assisted selection (MAS). After MAS in each generation, one of the F_5 plants was selected as a QTL-NIL for the target QTL.

Field evaluation of the QTL-NIL

Thirty plants of the selected QTL-NIL and Koshihikari were grown in the field in 2002. The germinated seeds were sown on May 16, and the seedlings were transplanted to the field on June 12. At 10 days after heading, the length of the culm and the second longest panicle were measured on five plants per line. Simultaneously, the frequency of the exserted stigma was also investigated using the same procedure described above.

Results

Genome constitution of 98SQ1496

The graphical genotype of 98SQ1496 is shown in Fig. 2. Thirteen chromosomal regions of IR24 were included in the genome. One substituted region on chromosomes 1, 5, 7, 8 and 9, two regions on chromosome 3 and three on chromosome 2 were homozygous for the IR24 allele. However, one region on chromosomes 3 and 10, and two regions on chromosome 12 remained heterozygous.

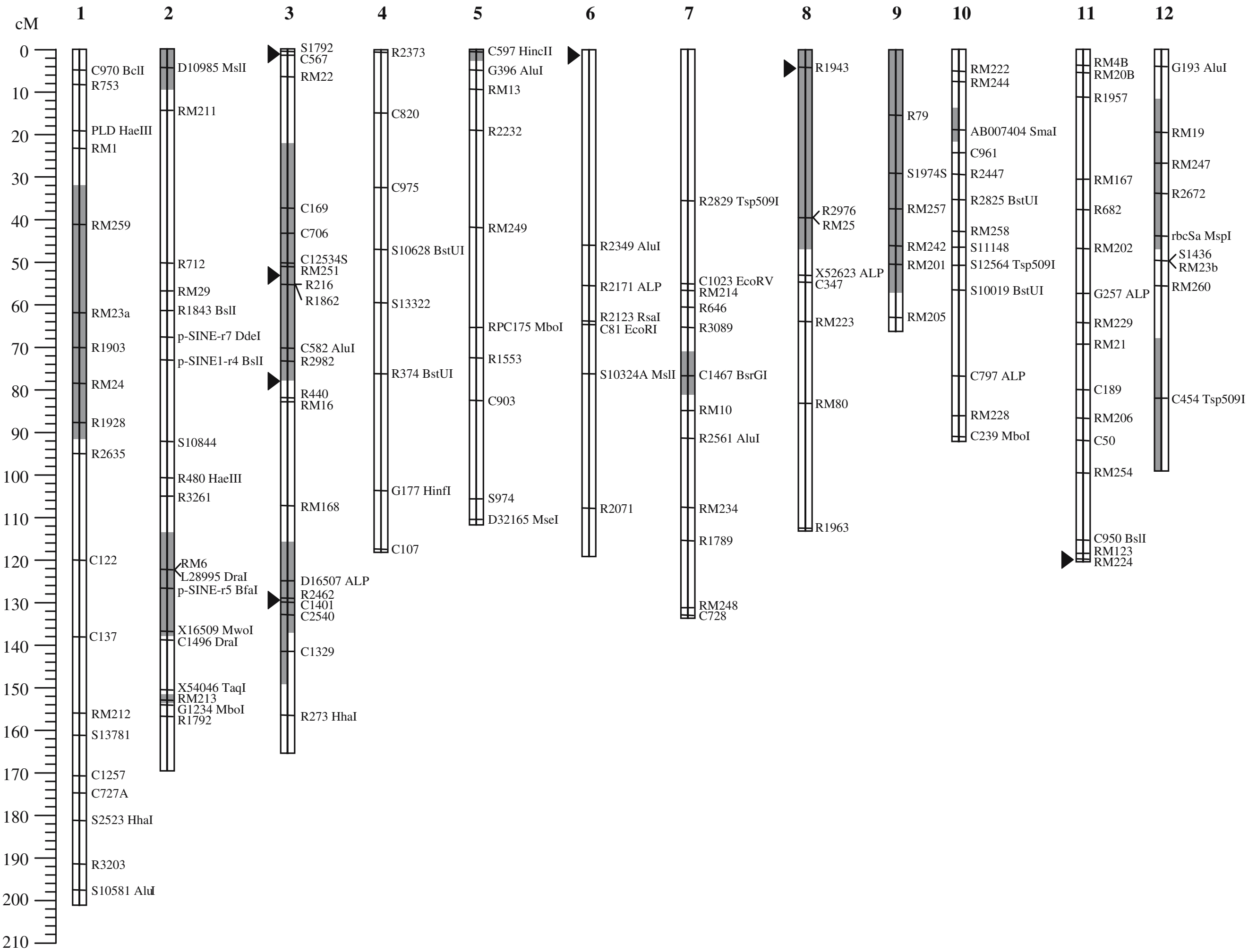


Fig. 2 Graphical genotype of 98SQ1496 (BC_1F_4). Markers are indicated to the *right* of each chromosome. *White* and *gray* regions represent the chromosomal segments of japonica (Koshihikari or Hoshinohikari) and IR24, respectively. *Triangles* correspond to the positions of the QTLs estimated in RILs: R1468B, XNpb238, R1002 and C1468 from short to long arm on chromosome 3, C1003B on chromosome 6, C277 on chromosome 8 and XNpb52 on chromosome 11 (Yamamoto et al. 2003)

Assuming that all the markers are equally distributed, 25% of the total genome in 98SQ1496 is derived from IR24. Among the seven regions of QTL increasing the rate of the exserted stigma at the IR24 allele detected in RIL (Yamamoto et al. 2003), four [XNpb238 (50.9 cM from distal end of the short arm), R1002 (82.5 cM) and C1468 (118.2 cM) on chromosome 3 and C277 (1.5 cM) on chromosome 8] are predicted to have remained in this plant.

Frequency distribution of stigma exsertion and correlation of other traits in F_2 population

The frequency distribution of the stigma exsertion rate in the F_2 population showed a continuous variation (Fig. 3). This suggests that a high capability of stigma exsertion in 98SQ1496 was controlled by multiple loci. Also, judging from the wide variation in the F_2, the same as that in the RILs (Yamamoto et al. 2003), an

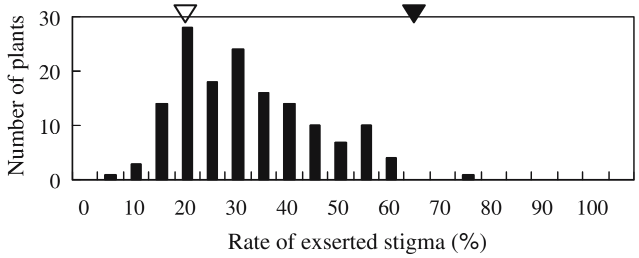


Fig. 3 Frequency distribution of the rate of stigma exsertion in F_2 population of the cross between Koshihikari and 98SQ1496. *White* and *black triangles* indicate the average values of Koshihikari and IR24, respectively

effective QTL appeared to exist under the genetic background close to the japonica rice.

Table 1 shows the phenotypic correlations among all the examined traits. The rate of the exserted stigma was positively correlated with the grain length and length–width ratio of the grain, while it was negatively correlated with the grain width. The variations in the heading date and panicle length are independent of the expression of the stigma exsertion.

QTL analysis

One prominent QTL was detected for the rate of exserted stigma on chromosome 3 (Fig. 4, Table 2). This QTL, designated *qES3*, explained 31.63% of the total phenotypic variance, and the IR24 allele at the QTL increased the rate of exserted stigma by 20.10%.

The QTL analysis for days to heading, panicle length and grain size (length, width and length–width

Table 1 Correlation coefficients among six traits in F_2 generation

Trait	ES	DTH	PL	GL	GW	GL/GW
Rate of exserted stigma (ES)						
Days to heading (DTH)	0.019					
Panicle length (PL)	0.112	−0.691 **				
Grain length (GL)	0.504 **	−0.174 *	0.243 **			
Grain width (GW)	−0.194 *	−0.293 **	0.238 **	0.253 **		
Ratio of grain length to grain width (GL/GW)	0.597 **	0.046	0.055	0.729 **	−0.477 **	

*, **Significant at $P < 0.05$ and $P < 0.01$, respectively

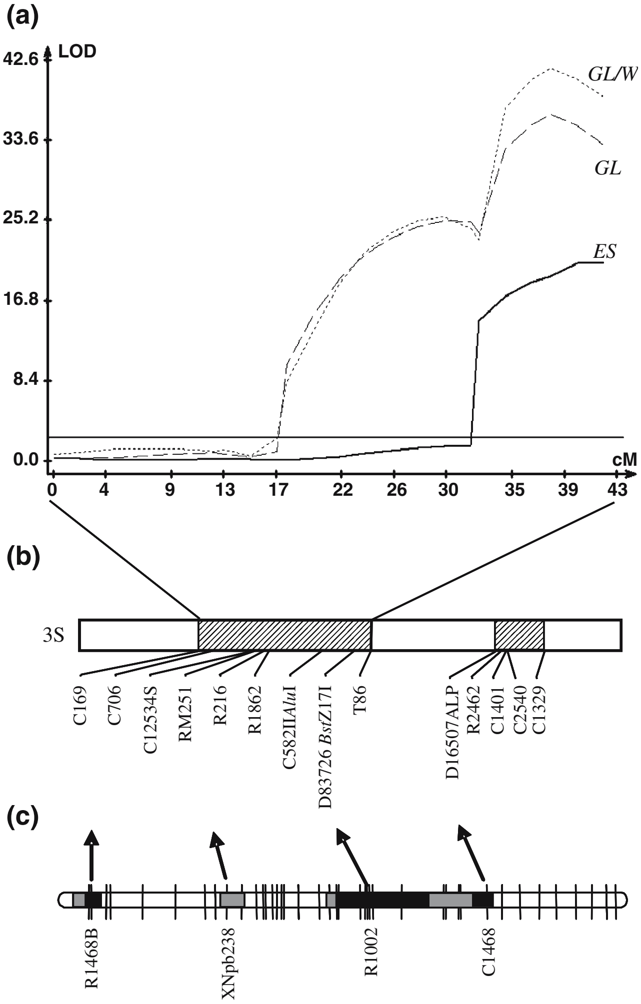


Fig. 4 **a** LOD score of QTLs for stigma exsertion (ES), grain length (GL) and ratio of grain length to grain width (GL/W) on chromosome 3. **b** Molecular linkage map of the F_2 population, Koshihikari/98SQ1496. *Striped boxes* indicate heterozygous regions. **c** QTLs detected in RILs of Asominori/IR24. *Black* and *gray boxes* indicate the regions with $F > 10.0$ and $F > 5.0$, respectively (Yamamoto et al. 2003)

Table 2 Putative QTLs for six traits detected in F_2 population between Koshihikari and 98SQ1496

Trait	QTL	Chromosome	Interval	Source	LOD	$R^{2\,a}$	*P*	AE^b
Rate of exserted stigma (%)	*qES3*	3	D83726 *Bst*Z17I -T86	IR24	18.05	31.63	0.0000	20.10
Days to heading (days)	*qDTH3*	3	C2540-C1329	IR24	12.17	25.90	0.0000	11.99
	qDTH8	8	R1943-R2976	Koshihikari	22.65	22.92	0.0000	9.25
Panicle length (cm)	*qPL8*	8	R1943-R2976	IR24	5.79	16.89	0.0000	1.09
	qPL9	9	R79-RM257	Koshihikari	4.02	11.18	0.0000	0.94
Grain length (mm)	*qGL3*	3	D83726 *Bst*Z17I -T86	IR24	17.45	17.46	0.0000	0.16
	qGL9	9	RM257-RM242	Koshihikari	3.99	11.09	0.0000	0.13
Grain width (mm)	*qGW2*	2	RM240-Wa590*Mwo*I	Koshihikari	6.79	9.83	0.0000	0.04
	qGW3	3	C706-C12534S	Koshihikari	4.80	5.69	0.0000	0.04
Length-width ratio of grain	*qGL/W3*	3	D83726 *Bst*Z17I -T86	IR24	13.69	8.96	0.0000	0.06

[a] Percent phenotypic variance explained by each QTL

[b] Additive effect of source allele

ratio) was also conducted (Table 2). Two QTLs (*qDTH3* and *qDTH8*) for days to heading were detected on each of chromosomes 3 and 8. *qDTH3* explained 25.90% of the total phenotypic variance, and the IR24 allele increased days to heading by about 12 days. *qDTH8* explained 22.92% of the total phenotypic variance, and the Koshihikari allele increased days to heading by about 9 days. Two QTLs (*qPL8* and *qPL9*) for panicle length were detected on each of chromosomes 8 and 9. *qPL8* explained 16.89% of the total phenotypic variance, and the IR24 allele increased the panicle length about 1.1 cm. *qPL9* explained 11.18% of the total phenotypic variance, and the Koshihikari allele increased the panicle length by about 0.9 cm.

Five QTLs (*qGL3*, *qGL9*, *qGW2*, *qGW3* and *qGL/W3*) related to grain shape were detected on chromosomes 2, 3 and 9. *qGL3* and *qGL/W3*, both of which increased the values at the IR24 allele, explained 17.46 and 8.96% of the total phenotypic variance, respectively. Judging from their similar positions and phenotypic direction, *qGL3* is predicted to have the same locus as *qGL/W3* (Fig. 4). On the other hand, the Koshihikari allele at *qGL9* increased the grain length with an 11.09% phenotypic variance. *qGW2* and *qGW3* for grain width explained 9.83 and 5.69% of the total phenotypic variance, respectively. The Koshihikari allele at these QTLs increased the grain width.

Evaluation of QTL-NIL for *qES3*

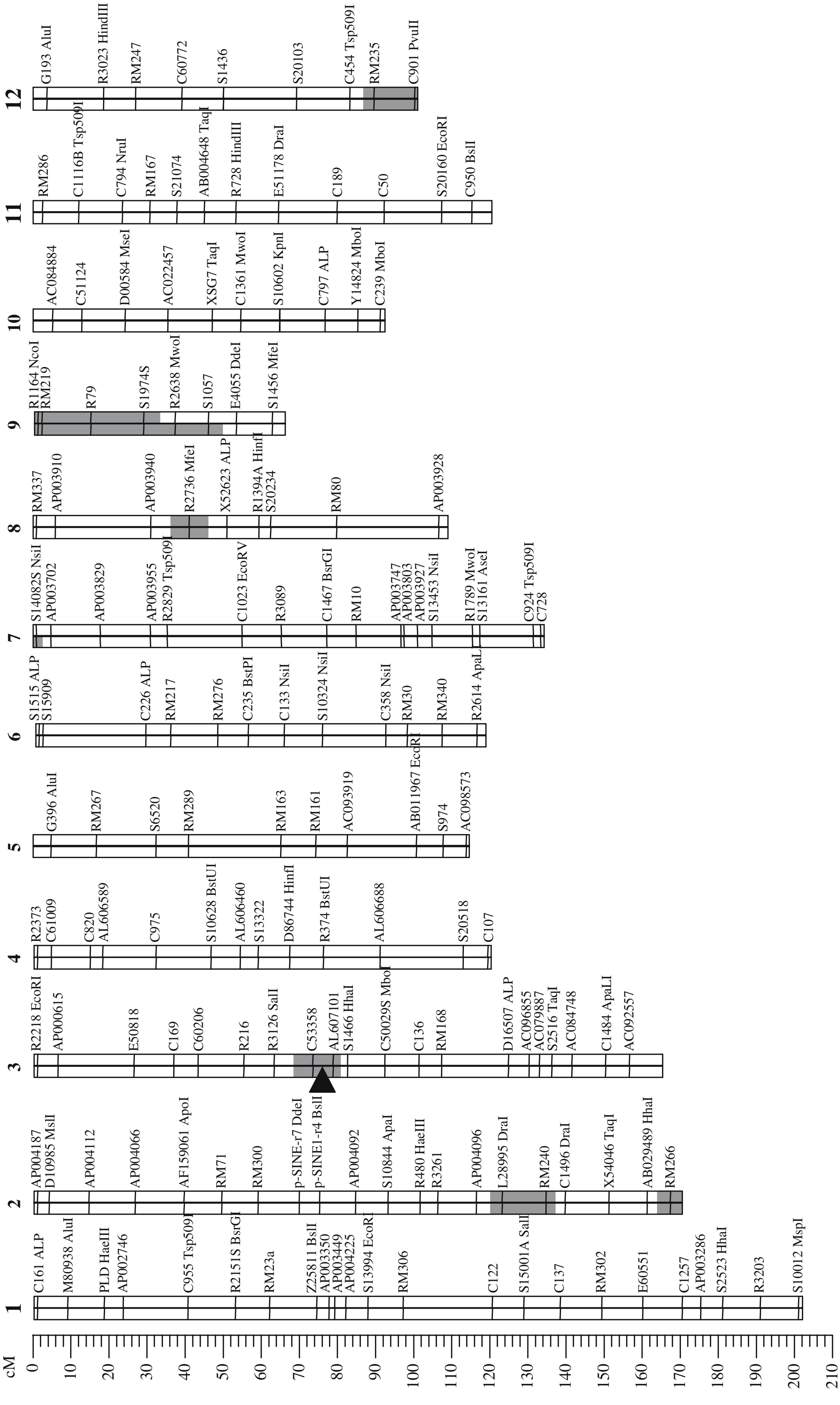
QTL-NIL possessing the IR24 segment in the region covering *qES3* [QTL-NIL (*qES3*)] was developed in the japonica genetic background. QTL-NIL (*qES3*) still keeps a total of six segments derived from IR24 on chromosomes 2 (two regions), 7, 8, 9 and 12 (Fig. 5). These results suggest that 98SQ1496 also possesses the chromosome region of IR24 in the distal end on chromosome 7 in addition to the 13 regions mentioned above. Figure 6 represents the rate of the exserted stigma, the culm length, the panicle length and the heading date of Koshihikari and QTL-NIL (*qES3*) during field cultivation. The rate of stigma exsertion was 50.5% in QTL-NIL (*qES3*) which was much higher than that of Koshihikari (13.1%). The appearance of exserted stigmas in QTL-NIL (*qES3*) is similar to that of IR24, the donor of the traits. The culm length of QTL-NIL (*qES3*) was 9.4 cm shorter than Koshihikari. There was no significant difference in the panicle length between QTL-NIL (*qES3*) and Koshihikari. The days to heading were shortened in QTL-NIL (*qES3*) by 6 days compared to that in Koshihikari.

Discussion

Comparison with QTL analysis in RILs

Yamamoto et al. (2003) reported that seven QTLs on chromosomes 3, 6, 8 and 11 were responsible for increasing the rate of the exserted stigma at the IR24 allele by using RILs: four QTLs on chromosome 3 and one QTL on each of chromosomes 6, 8 and 11. Among them, three on chromosome 3 (XNpb238, R1002 and C1468) and one on chromosome 8 (C277) are still predicted to be kept as the IR24 allele in 98SQ1496. A large phenotypic effect was detected at *qES3*, located in the centromeric region (R1002) on chromosome 3, in both the QTL analyses using the RILs and the F_2. While the phenotypic variance explained by *qES3* was 16.96% in the RILs, it was 31.63% in the F_2, indicating that the QTL effect would be more prominently

Fig. 5 Graphical genotype of QTL-NIL (*qES3*). Markers are indicated to the *right* of each chromosome. *White* and *gray* regions represent the chromosomal segments of japonica varieties (Koshihikari or Hoshinohikari) and IR24, respectively. *Triangles* correspond to the positions of *qES3* ➜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C161 ALP
M80938 AluI
PLD HaeIII
AP002746
C955 Tsp509I
R2151S BsrGI
RM23a
Z25811 BslI
AP003350
AP003449
AP004225
S13994 EcoRI
RM306
C122
S15001A SalI
C137
RM302
E60551
C1257
AP003286
S2523 HhaI
R3203
S10012 MspI
2
AP004187
D10985 MslI
AP004112
AP004066
AF159061 ApoI
RM71
RM300
p-SINE-r7 DdeI
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AP004092
S10844 ApaI
R480 HaeIII
R3261
AP004096
L28995 DraI
RM240
C1496 DraI
X54046 TaqI
AB029489 HhaI
RM266
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E50818
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C60206
R216
R3126 SalI
C53358
AL607101
S1466 HhaI
C50029S MboI
C136
RM168
D16507 ALP
AC096855
AC079887
S2516 TaqI
AC084748
C1484 ApaLI
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C226 ALP
RM217
RM276
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C133 NsiI
S10324 NsiI
C358 NsiI
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C1467 BsrGI
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AP003803
AP003927
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C728
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C797 ALP
Y14824 MboI
C239 MboI
11
RM286
C1116B Tsp509I
C794 NruI
RM167
S21074
AB004648 TaqI
R728 HindIII
E51178 DraI
C189
C50
S20160 EcoRI
C950 BslI
12
G193 AluI
R3023 HindIII
RM247
C60772
S1436
S20103
C454 Tsp509I
RM235
C901 PvuII

observed under the japonica genetic background. These results revealed that the IR24 allele at *qES3* expressed the phenotype for the stigma exsertion with the Koshihikari genetic background. Even though there are three additional QTLs detected in the previous analysis and considered to segregate in this F_2, which may be the reason for the continuous variation of this trait (Fig. 3), no other significant QTLs were detected in this analysis. One of the three QTL (C1468) on the long arm on chromosome 3 was just slightly under the significant level in this analysis (data not shown). Since the remaining two, one on chromosome 3 (XNpb238) and the other on chromosome 8 (C277), are predicted on the border region of the IR24 segment in 98SQ1496, these may have been substituted to the Koshihikari homozygous. Otherwise, their effects are masked by an epistatic interaction with some other QTLs.

Correlation between stigma exsertion and other floral traits

The frequency of the exserted stigma positively correlated with the grain length and the length–width ratio of the grain. This is consistent with previous reports illustrating the positive correlation between stigma exsertion and pistil length, stigma length, spikelet length or the length–width ratio of the spikelet (Kato and Namai 1987a, b; Virmani and Athwal 1973, 1974). A QTL analysis detected the putative QTLs in the centromeric region on chromosome 3 for stigma exsertion (*qES3*), grain length (*qGL3*) and length–width ratio of grain (*qGL/W3*) (Fig. 4). Several independent studies have also identified QTLs associated with the pistil or grain in this region. For example, QTLs for the pistil length or style length have been reported in RILs from Asominori and IR24 (Uga et al. 2001), and QTLs for grain weight or length in different populations (Brondani et al. 2002; Kubo et al. 2001; Li et al. 1997, 2004a, b; Moncada et al. 2001; Redoña and Mackill 1998; Thomson et al. 2003; Xiao et al. 1998; Xing et al. 2002; Yu et al. 1997). The adjacency of these QTLs suggests correlations between the stigma exsertion and the other pistil or spikelet traits. To confirm whether these correlations results from a tight linkage or pleiotropic effect, a further analysis will be necessary. Although the mechanism underlying stigma exsertion remains to be elucidated, it is relevant that stigma expansion results in an increased frequency of stigma exsertion on the grounds that longitudinal elongation of the reproductive organs can account for the above traits on the pistil and spikelet.

QTL-NIL for *qES3* as maternal line of hybrid rice

For the purpose of breeding a suitable stigma exserted line, we tried to improve the poor traits of IL223, which had been selected by conventional breeding, through QTL analysis and marker assisted selection. Field evaluation of QTL-NIL (*qES3*) compared with Koshihikari, the recurrent line, pointed out that several differences described as follows still remain: (1) QTL-NIL (*qES3*) showed an earlier heading probably due to the influence of *qDTH8*, which was mapped to the similar chromosomal position of *dthA8a* (Kubo et al. 2002). (2) QTL-NIL (*qES3*) was not as long as Koshihikari in culm length. Its difference seems to be due to the pleiotropic effect of *dthA8a*, since some studies reported a correspondence between QTLs for heading date and plant height (Li et al. 1995; Xiao et al. 1995, 1996). (3) Grain shape remains to be improved in QTL-NIL (*qES3*). It would be difficult to break the correlation between the stigma exsertion and grain length, if these traits are controlled by a single locus. In conclusion, the differences in the heading date and culm length in QTL-NIL (*qES3*) could be improved by the substitution to japonica allele in *qDTH8*. However, a further analysis will be needed for the separation of the grain shape trait and stigma exsertion.

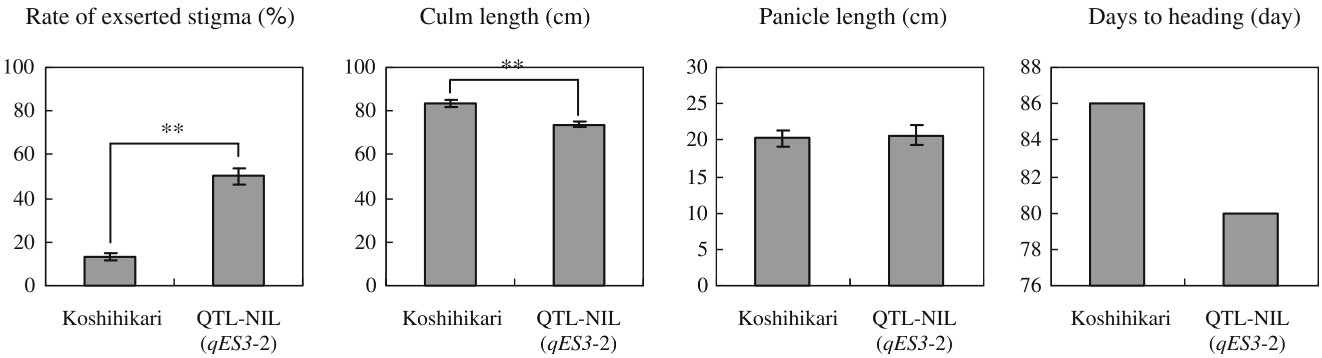

Fig. 6 Agricultural traits of QTL-NIL (*qES3*). *Asterisks* indicate significant levels at $P < 0.01$

Our results demonstrated the usefulness of DNA markers for the practical breeding of one of the floral traits in rice. Uga et al. (2003) reported the QTLs for the rate of exserted stigma on chromosomes 5 and 10, stigma length on chromosomes 4 and 6 and stigma breadth on chromosomes 5 and 10 in RILs between Pei-Kuh and *Oryza rufipogon*. All of these QTLs were mapped to distinct locations from *qES3*. Although the effect of the combined QTLs from a diverse germplasm is uncertain for stigma exsertion, these QTLs may be useful as parts of the QTL pyramiding for making highly allogamous-like stigma in rice plants which would increase the seed production in hybrid rice.

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