Research Paper

Detection of dominant QTLs for stigma exsertion ratio in rice derived from *Oryza rufipogon* accession ‘W0120’

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Stigma exsertion can enhance the outcrossing fertility in rice. Dominant genes for this trait are expected to be essential for the effective application of a novel outcrossing-based breeding system that uses male sterility in rice, which is normally autogamous. Because reduction of stigma exsertion is a domestication trait, we screened wild rice species as possible donors of genes or QTLs for stigma exsertion. We used *in silico* image-based screening and selected the *Oryza rufipogon* accession ‘W0120’. A single F₁ individual derived from a cross between the *japonica* rice cultivar ‘Akidawara’ and ‘W0120’ was used to generate F₂ and BC₁F₁ populations. QTL analysis performed using 114 F₂ individuals detected QTLs on chromosomes 2, 3, 4, 8, and 11. Only two major QTLs on chromosomes 3 and 8 showed higher degrees of dominance. On the other hand, there were no QTLs near *GS3*, which is well known as a gene for stigma exsertion. Validation of these QTLs using 188 BC₁F₁ individuals provided clear evidence for their dominance. Genotypes of the markers nearest to the two QTLs were also related to grain length. We expect the genes responsible for these QTLs to be promising tools for improving outcrossing-based breeding in rice.

Key Words: dominant QTL, male sterility, outcrossing fertility, *Oryza rufipogon* Griff., outcrossing fertility, rice (*Oryza sativa* L.), stigma exsertion.

Introduction

In conventional breeding of autogamous crops such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and soybean (*Glycine max*), breeders generally use pedigree breeding (Brescaghiello and Coelho 2013). They cross two materials, then perform genetic fixation by selfing, phenotype-based selection, and evaluation of regional adaptability. Finally, the selected line is registered as a cultivar. This procedure requires approximately one decade and the typical number of usable materials is two. To breed the best possible cultivar, breeders must cross the top cultivars. After several decades, repeating this breeding cycle decreases the genetic diversity of the breeding population and therefore the breeding effect. Indeed, the yield improvements of autogamous crops have stagnated after the 1990s (Tanaka and Tabei 2014).

In contrast, the increase in the yield of maize, an alloga-

mous crop, due to breeding has still not leveled off (USDA National Agricultural Statistics Service, <https://www.nass.usda.gov/index.php>). Maize cultivars are usually F₁ hybrids derived from crossing between two parental lines. The increase in the yield of maize is driven mainly by the improvement of parental lines by recurrent selection (RS)-based breeding, rather than by finding new crossing combinations for new F₁ hybrid cultivars. In RS, maize breeders repeat the cycles of outcrossing and selection after selfing in breeding populations. Multiple successive rounds of selection increase the frequency of desirable combinations of alleles and improve the genetic gain in the breeding population. Breeders can use many breeding materials (founders), and there is frequent outcrossing among them. Thus, RS-based breeding continuously improves maize yield.

To implement the maize-like breeding methods for an autogamous crop, recurrent selection using genetic male sterility has been proposed and attempted to practice in autogamous crop breeding (Brim and Stuber 1973, Doggett and Eberhart 1968, Fujimaki 1980). However, these breeding systems have not become a major breeding method in autogamous crops because they require much additional labor. As a more efficient system, recurrent selection using

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transgenic male sterility (RSUTMS) was proposed (Tanaka 2010). RSUTMS is based on the development and application of dominant transgenic male sterility (TMS) with positively and negatively selectable marker traits. A dominant TMS gene cassette facilitates efficient outcrossing without manual emasculation, while the positively and negatively selectable marker gene cassettes allow to distinguish between male-sterile and fertile individuals. The introduction of such gene cassettes can create a positively and negatively selectable TMS plant to facilitate RS in autogamous crops.

Some TMS rice lines have been developed (Abe *et al.* 2018, Akasaka *et al.* 2018, Lu *et al.* 2000), but their flowering habits are not suitable for RS-based breeding because of their delayed heading, flowering, and daily flowering time relative to the wild type (Abe *et al.* 2018). Similar delays were observed in cytoplasmic male sterility (MS) in hybrid rice, which caused failure of opening of 20% of female parent spikelets (Yan and Li 1987), partial or full panicle enclosure (Singh and Shirisha 2003, Tien *et al.* 2013, Yin *et al.* 2007), and asynchronous flowering (Lou *et al.* 2014). These features decrease outcrossing fertility.

The development of a gene cassette to increase outcrossing fertility of plants with dominant TMS would provide a useful tool for the novel breeding system in rice. Plants with dominant TMS cannot produce viable pollen grains and therefore require wild-type pollen for fertility. For this reason, all individuals with dominant TMS must be heterozygous for the TMS gene cassette. Consequently, the newly developed gene cassette to increase outcrossing fertility must be dominant when introduced together with the TMS gene cassette by the same vector into the same locus. If the new cassette is recessive, it will not be functional with dominant TMS.

A dominant gene could be under control of a native promoter, or the promoter could be improved, for example, to increase the expression level and to narrow the tissue specificity. In contrast, the use of a recessive gene must be combined with RNA interference technology or similar techniques; however, it is widely recognized that it is difficult to perfectly suppress the gene activity by these techniques, and the effectiveness of these techniques tends to be unstable in long-term applications (Mitsuhashi *et al.* 2002). The availability of a dominant cassette to increase rice outcrossing fertility will make RSUTMS more efficient and applicable to large-scale rice breeding.

Stigma exertion can improve outcrossing fertility in rice (Kato and Namai 1987). The exerted stigma can stay viable and accept pollen for 6 days (Xu and Shen 1987, Yan and Li 1987, Yan *et al.* 2009). This trait increases the opportunity for pollination in female parents of hybrid cultivars (Kato and Namai 1987) and overcomes the barrier of non-synchronous flowering between the parents (Lou *et al.* 2014). This trait is used for the improvement of hybrid rice seed production (Takano-Kai *et al.* 2011, Yan *et al.* 2009) because it increases the opportunity for pollination in female parents of hybrid cultivars (Kato and Namai 1987) and

overcomes the barrier of non-synchronous flowering between the parents (Lou *et al.* 2014).

As a quantitative trait, stigma exertion is controlled polygenically and has no cytoplasmic effect (Li and Chen 1985, Virmani and Athwal 1974). It has high heritability and can be improved by breeding (Virmani 1994, Yan *et al.* 2009). Many QTLs for this trait have been reported (Li *et al.* 2014, Lou *et al.* 2014, Miyata *et al.* 2007, Rahman *et al.* 2016, Uga *et al.* 2003, Yamamoto *et al.* 2003). The most common and most powerful QTL for stigma exertion is frequently detected near the centromere in the short arm of chromosome 3 (Li *et al.* 2014, Miyata *et al.* 2007, Yamamoto *et al.* 2003). The responsible gene of this QTL has been cloned as the *Grain Size 3* gene (*GS3*) (Fan *et al.* 2006, Takano-Kai *et al.* 2009, 2011). *GS3* is a major gene for seed length (Fan *et al.* 2006); it also affects stigma length and stigma exertion in rice (Takano-Kai *et al.* 2011), but the long and narrow grain and stigma exertion-type allele of *GS3* is recessive.

Reduction of stigma exertion is a domestication trait in rice (Huang *et al.* 2012). In wild rice species, stigma exertion facilitates outcrossing and maintains the diversity of natural populations. This diversity plays a key role in adaptation to environmental fluctuations as a wild species. On the other hand, stigma exertion is not desirable in cultivars because it disturbs the uniformity and stability achieved by selfing. Generally, during domestication, many functional (dominant) alleles of wild species mutate to loss-of-function (recessive) alleles. Wild relatives would preserve the dominant alleles and would thus seem to be promising resources for detection of such alleles, including those for stigma exertion.

There is a wide range of variability for stigma exertion among wild rice species (Marathi and Jena 2015, Uga *et al.* 2003). In this study, we screened wild rice species and detected two clearly dominant QTLs for stigma exertion, which have the potential to improve RSUTMS in the future.

Materials and Methods

Database screening of donor accessions

To obtain suitable genetic material for QTL analysis, we searched the Oryzabase database (<https://shigen.nig.ac.jp/rice/oryzabase/>) using 23 image files of the flowers of wild rice accessions on the “Flower” page under “Image Gallery”. The screening criteria were as follows:

- 1) Clear stigma exertion.
- 2) More closely related to *O. sativa* to give lower crossing isolation and more frequent genetic recombination.
- 3) Rounder grain shape, which is desirable without long grain type of the allele of *GS3* as a background at the QTL analysis to detect the other QTLs.

Construction of populations for genetic analysis

Because we aimed to detect dominant QTLs, genetically fixed materials were not suitable, so we prepared F₂ and BC₁F₁ populations. The F₁ individuals were generated by

crossing the *japonica* cultivar ‘Akidawara’ and *O. rufipogon* accession ‘W0120’ in a simplified biotron breeding system (Tanaka *et al.* 2016). Because wild rice, especially the perennial types such as *O. rufipogon*, has a high probability of outcrossing in nature (Huang *et al.* 2012, Khush 1997), ‘W0120’ might have heterozygous genome regions. Therefore, only one F₁ individual was arbitrarily selected and selfed to produce the F₂ population for QTL analysis. The BC₁F₁ population, used to validate the detected dominant QTLs, was derived by backcrossing ‘Akidawara’ with the same F₁ plant used for the preparation of the F₂ population.

Phenotypic evaluation of stigma exertion

Because wild species and their progeny generally have deep seed dormancy, all seeds were soaked in 1% H₂O₂ for 2 days before sowing. Seedlings were grown in nursery boxes for 4 weeks, and each individual was transplanted into a horticultural pot and grown in summer in a NARO research field in Kannondai, Tsukuba, Ibaraki, Japan (36°13'N, 140°64'E). To synchronize heading as much as possible, short-day treatment was applied in the biotron for 2–3 weeks.

Stigma exertion was evaluated at 7–10 days after heading in all spikelets of one spike per individual. Spikelets with stigma exertion and total spikelets were counted, and the ratio of spikelets with stigma exertion to total spikelets (stigma exertion ratio, SER, %) was calculated.

DNA marker detection

Total DNA was extracted from a young leaf (3–4 weeks old) of each individual with a DNA *Sui Sui-S* extraction kit v. 1.01 (Rizo Inc., Tsukuba, Japan). Indel markers were used because they are codominant, have high density, and are convenient for direct detection by PCR (Liu *et al.* 2013, Păcurar *et al.* 2012). All markers were chosen from among those listed in Yonemaru *et al.* (2015) with consideration of the GC content of the amplicon, melting temperature of primers, and marker position in the rice genome. In total, 216 markers were tested for their polymorphisms, and markers polymorphic between the parental accessions were used for genotyping.

PCR was performed in a final volume of 10 µl containing 5 µl GoTaq Green Master Mix (Promega Corporation, Madison, WI, USA), 5 µM each of a PCR-primer pair, 0.1 µM each of a PCR-primer pair and 2 ng of template DNA. Touchdown PCR was performed as follows: initial denaturation for 3.5 min at 94°C; 34 cycles of 30 s at 94°C, 60 s at annealing temperature (described below), and 30 s at 72°C; 10 min at 72°C. The annealing temperature was 62°C in the first cycle; lowered by 0.5°C per cycle during cycles 2 to 14; and retained at 55°C for the last 20 cycles. PCR products were electrophoresed in agarose gel in TAE buffer and stained with ethidium bromide.

Construction of a linkage map and QTL analysis

The F₂ population included 114 individuals. A linkage

map was constructed in Antmap v. 1.2 software (Iwata and Ninomiya 2006). Grouping of markers into linkage groups was performed using the nearest-neighboring locus method with a threshold grouping criterion of 0.4. Genetic distances were calculated using the Kosambi mapping function (Kosambi 1944). QTL analysis was performed in Windows QTL Cartographer 2.5 software (Wang *et al.* 2006) using composite interval mapping with a manual threshold value of LOD = 2.0. Each detected QTL was named according to QTL nomenclature (McCouch *et al.* 1997).

QTL validation

In the BC₁F₁ population (188 individuals), marker genotypes were expected to be either homozygous for the ‘Akidawara’ type or heterozygous between ‘Akidawara’ and the donor. The expected segregation ratio was 1:1. A *t*-test was used to check the significance of differences in SER between genotypes of the flanking markers in the BC₁F₁ population. ANOVA and Tukey’s honestly significant difference (HSD) test were used for multiple comparisons between genotype combinations. The dominant effect of QTLs, calculated as the ratio of the average phenotypic value of heterozygotes to the average phenotypic value of both parents, was evaluated using the genotypes of markers nearest to the QTLs detected in the F₂ population.

Evaluation of the shape of BC₁F₂ grains

BC₁F₂ seeds were harvested from 116 BC₁F₁ individuals and used for grain shape evaluation. The length, width, and ratio of length to width of all grains of each line were measured in SmartGrain v. 1.1 software (Tanabata *et al.* 2012).

Results

Database screening of donor accessions

Among 23 images of the flowers of wild rice accessions available in Oryzabase, 19 images were suitable for evaluation of the level of stigma exertion. By eye, we selected 8 accessions with clear stigma exertion (Table 1). They included two accessions (‘W0120’ and ‘W1945’) of *O. rufipogon*, which is most closely related to *O. sativa*. We chose ‘W0120’ for its rounded grain shape and clear stigma exertion.

Table 1. Wild rice accessions with stigma exertion identified from flower images in the Oryzabase database

Acc. No.	Species	Genome	Country of origin	Grain shape
W0001	<i>O. ridleyi</i>	HHJJ	Thailand	Slender
W0120	<i>O. rufipogon</i>	AA	India	Somewhat slender
W1185	<i>O. glumaepatula</i>	AA	Suriname	Somewhat slender
W1194	<i>O. grandiglumis</i>	CCDD	Brazil	Sharp-pointed
W1197	<i>O. latifolia</i>	CCDD	Colombia	Sharp-pointed
W1413	<i>O. longistaminata</i>	AA	Sierra Leone	Somewhat slender
W1945	<i>O. rufipogon</i>	AA		Slender
W2199	<i>O. glumaepatula</i>	AA	Brazil	Slender

Stigma exertion ratio of 'W0120' and the F_1 plant

The SER of 'W0120' was 89.3% (average of 11 individuals), and the SER of a F_1 plant derived from a cross between 'W0120' and 'Akidawara' was 64.5% (average of 7 spikes). On the other hand, the SER of 'Akidawara' was 18.8% (average of 20 plants).

Linkage mapping and QTL analysis

Of the 216 markers evaluated, 105 were polymorphic; of these, we selected 92 on the basis of their polymorphisms and distribution (Supplemental Table 1). The SER in the F_2 population showed a continuous variation from 0% to

90% (average, 36%).

The linkage map included 12 linkage groups and 92 markers with no gaps; the total length was 1555 cM (Fig. 1). Five QTLs for SER were detected in the F_2 population on chromosomes 2, 3, 4, 8, and 11 (Fig. 1, Table 2). Except for the QTL on chromosome 4, the 'W0120' alleles increased SER. Two major QTLs were detected: $qSER3$, near the end of the short arm of chromosome 3, and $qSER8$, on the short arm of chromosome 8. Both had stronger dominance effects than the other QTLs. Contributions to phenotypic variation were 17% by $qSER3$ and 13% by $qSER8$. The region of $GS3$, which is the most commonly detected

Fig. 1. Linkage map and the QTLs for stigma exertion ratio (SER) detected in the F_2 population derived from a cross between 'Akidawara' and 'W0120'. Arrows indicate QTLs whose 'W0120'-type alleles ↑ increase and ↓ decrease SER.

Table 2. QTLs for stigma exertion ratio detected in the F_2 population from a cross between 'Akidawara' and 'W0120'

QTLs	Chromosome	Position (cM)	LOD	$R^2(\%)^a$	Add. ^b	Dom. ^c	The Nearest Markers	Marker Position	Nearby previously detected QTL or the nearest marker to the peak
$qSER2$	2	6.0	2.0	9.8	10.1	1.4	C5-indel1361	827575	qPES-2 (Deng <i>et al.</i> 2010)
$qSER3$	3	0.8	3.2	14.5	5.9	13.5	C5-indel2400	537699	R1468B (Yamamoto <i>et al.</i> 2003)
$qSER4$	4	21.6	2.0	1.5	-12.1	6.4	C5-indel3632	5050622	
$qSER8$	8	12.5	2.3	12.6	6.6	11.6	C5-indel6873	1677212	C277 (Yamamoto <i>et al.</i> 2003)
$qSER11$	11	1.0	2.2	4.5	9.9	-4.5	C5-indel8795	855285	qSSE-11 qDSE-11 (Rahman <i>et al.</i> 2016) qTSE-11

^a Phenotypic variance explained by QTL.

^b Affected by additive effect.

^c Degree of dominance.

Fig. 2. Validation of the two dominant QTLs for stigma exsertion ratio (SER) in the BC₁F₁ population. (A) *qSER3* (C5-indel2400), (B) *qSER8* (C5-indel6873). ■ 'Akidawara' type; □ heterozygotes between 'Akidawara' and 'W0120'. ↓ SER of 'Akidawara'; ↑ SER of 'W0120'.

QTL region for stigma exsertion in rice (Li *et al.* 2014, Miyata *et al.* 2007, Yamamoto *et al.* 2003), was not detected in this population.

QTL validation

The frequency of the distribution of SER in the BC₁F₁ population varied from 0% to 89% (average, 27.8%). Genotypes of the markers nearest to the QTLs on chromosomes 3 (C5-indel2400) and 8 (C5-indel6873) were clearly related to the SER phenotype (Fig. 2), with *P*-values of 0.0001 and 0.001, respectively, by *t*-test. The dominant effect was 1.55 for C5-indel2400 and 1.43 for C5-indel6873. The theoretical segregation ratio in the BC₁F₁ population is 1:1, but homozygotes for the 'Akidawara'-type alleles of both markers appeared more often than heterozygotes, and the distortions of segregation ratio were significant. Individuals with the heterozygous genotypes of both *qSER3* and *qSER8* showed 38.9% SER (Supplemental Fig. 1).

Relationships between the major detected QTLs for SER and grain shape

Evaluation using BC₁F₂ seeds showed that C5-indel2400 and C5-indel6873 were related to grain length and the ratio of length to width in the BC₁F₁ population (Fig. 3), but not to grain width. We could not detect any clear relationship between SER and grain shape traits.

Discussion

Effectiveness of screening of wild rice species

Because the outcrossings promoted by stigma exsertion reduces the uniformity and sustainability of cultivars of autogamous crops, we assumed that genes related to this trait are easily lost during domestication. Because many genes related to the domestication mutate from dominant to recessive during domestication (Li *et al.* 2006), wild relatives

Fig. 3. Relationship between the genotypes of indel markers nearest to *qSER3* and *qSER8* and grain length in the BC₁F₂ seeds derived from BC₁F₁ in Fig. 2. (A) C5-indel2400, the nearest marker to *qSER3*; (B) C5-indel6873, the nearest marker to *qSER8*. ■ 'Akidawara' type; □ heterozygotes between 'Akidawara' and 'W0120'. ↓ SER of 'Akidawara'; ↑ SER of 'W0120'.

would seem to be a great reservoir of dominant genes. In this study, we screened wild relatives of rice and selected 'W0120' as an ideal accession with clear stigma exsertion. We expected that 'W0120' may have a major dominant QTL, but found that the trait was controlled by several QTLs, similar to previous reports (Li *et al.* 2014, Lou *et al.* 2014, Miyata *et al.* 2007, Rahman *et al.* 2016, Uga *et al.* 2003, Yamamoto *et al.* 2003).

An F₁ individual from a cross between 'W0120' and 'Akidawara' showed clear stigma exsertion, which indicated the existence of at least one clearly dominant QTL. In fact, we detected two such QTLs, but none in the *GS3* region in which Cai and Morishima (2002) detected a major QTL for grain shape. There is no polymorphism in the *GS3* exon between 'W0120' and *japonica*-type rice cultivars in TASUKE (Kumagai *et al.* 2013, <http://ricegenomes.dna.affrc.go.jp/>), but there are many polymorphisms in the promoters and introns. The absence of a QTL in the *GS3* region in our study indicates that these polymorphisms did not considerably affect *GS3* function. The absence of QTL detection in the *GS3* region must have increased the detectability of other QTLs. The screening of the materials with rounder shape as donor is assumed to be one of the reasons for the successful detection of QTLs outside of the *GS3* region.

Rice chromosomal segment substitution lines (CSSLs, Ebitani *et al.* 2005, Kubo *et al.* 2002, Takai *et al.* 2014) are excellent materials in which to detect QTLs. However, to detect new QTL alleles with large effects, starting from phenotype-based screening will be effective, rather than using the already-developed CSSLs.

Strategy to detect dominant QTLs using F2 and BC1F1 populations

The type of population considerably affects the results of

QTL analysis. In recent years, genetically fixed materials such as recombinant inbred lines (RILs) and CSSLs have often been used for QTL analysis of autogamous crops. The traits of these materials can be evaluated repeatedly with high reliability. However, genetically fixed materials are unsuitable for the detection of dominant QTLs. Although an F_2 population is suitable for producing a large number of populations and in which dominant effects can be evaluated, it is not suitable for high-sensitivity QTL analysis because evaluation using multiple individuals cannot be performed. On the other hand, production of a BC_1F_1 population requires a large number of crossings, but it can be used for more precise analysis of dominant QTLs because of the genome-wide distribution of heterozygous regions, which eliminates the effects of recessive QTLs, and because of the theoretical 1:1 segregation ratio. We chose a strategy that included an initial QTL analysis using an F_2 population and then QTL validation using a BC_1F_1 population. Using the F_2 population, we detected two QTLs with clear dominant effects but low LOD values, and validated them using a BC_1F_1 population. Thus, the above strategy was efficient for our purpose.

Relationship between the detected QTLs and grain shape

We detected *qSER3* and *qSER8*, two clearly dominant QTLs for stigma exsertion. *qSER8* was mapped near *qPES-8*, which was detected as a QTL for stigma exsertion using a doubled haploid population derived from *japonica* and *indica* varieties (Deng *et al.* 2010). Although *qSER8* and *qPES-8* were detected in different materials, they might be the same QTL. *qSER3* was detected almost at the end of the short arm of chromosome 3, where the QTL related to stigma exsertion was reported by Yamamoto *et al.* 2003. These two QTLs may be different or different alleles, because the previously detected QTL should have a very small degree of dominance (Yamamoto *et al.* 2003).

Both QTL regions related to stigma exsertion were also related to grain length in the BC_1F_1 population. It is unknown whether this is due to pleiotropic effects or to linkage. As *GS3* also affects both stigma exsertion and grain length, this gene and the two detected QTLs might affect stigma exsertion through a common mechanism. Near the *qSER3* region, *OsCDPK1* affects grain shape (Ho *et al.* 2013). *OsFIE1* (Folsom *et al.* 2014) and *OsFIE2* (Nallamilli *et al.* 2013), located near the *qSER8* region, also affect grain shape. However, these genes seem to be distinct from the QTLs detected in this study because they are located far from the peaks of our QTLs. In addition, *qSER3* and *qSER8* affect only the longitudinal axis direction of the grain, whereas the three previously identified genes influence grain size.

Potential application of detected QTLs to improving seed productivity of hybrid rice

Introducing the QTLs for stigma exsertion into the seed parents of hybrid rice cultivars by backcrossing with

‘W0120’ as a donor can improve hybrid seed productivity. However, because the effect is dominant, the seed parent trait will be expressed in F_1 cultivars. In introducing the QTLs into the seed parents of hybrid cultivars, breeders should pay attention to influencing the stigma exsertion ratio of hybrid cultivars. Also, if the introduced QTLs are pleiotropic, they will affect the grain shape of F_1 cultivars. Although long grain might increase yield, recessive genes such as *GS3* may be easier to use for improving seed productivity without changing the grain traits of F_1 cultivars.

Toward application of detected dominant QTLs to a new rice breeding method

qSER3 and *qSER8* may be used to increase seed productivity in RSUTMS through cloning of the respective genes via fine mapping and improving the flowering habit. These genes can be used with native promoters, but to improve stigma exsertion only in male-sterile individuals, an effective pistil-specific promoter may be needed, which can be identified using a gene expression profile database (Akasaka *et al.* 2018). Using one of the two genes may be sufficient to improve stigma exsertion.

For RS in autogamous crop species, an efficient outcrossing system is required, but the flowering characteristics of rice are not suitable for efficient outcrossing, especially in temperate *japonica* cultivars. The constructs for RSUTMS contain a dominant MS gene cassette and positively and negatively selectable marker trait gene cassettes. The constructs can potentially be improved by introducing additional gene cassettes. Detection and application of genes that make autogamous crops allogamous such as those detected in this study can improve the flowering habit of MS rice plants for RSUTMS.

In many crop breeding programs, a reduction in the diversity of breeding populations limits breeding effects, even in the era of advanced genome analysis technology. To overcome this limitation, an efficient outcrossing-based breeding system is required. We expect that MS with a flowering habit improved by a QTL detected in this study will be useful for RSUTMS and will help to overcome the limitation of autogamous rice breeding.

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