

**Detection of QTLs for stigma exertion ratio for improving recurrent
selection using transgenic male sterility (RSUTMS) system of rice**

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using transgenic male sterility (RSUTMS) system of rice**

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Summary

Improvement of the yield of autogamous crops such as rice (*Oryza sativa*) and wheat (*Triticum aestivum*) through a conventional breeding method like pedigree breeding was reported has been stagnated since the 1990s. In contrast, the increased yield of maize, an allogamous crop, due to recurrent selection (RS)-based breeding has not leveled off. A novel breeding method namely recurrent selection using transgenic male sterility (RSUTMS) has been proposed for rice to implement the maize-like breeding method for an autogamous crop. Transgenic male sterile (TMS) rice lines were developed; however, their flowering habit was not good compared to the wild-type. Introduction of stigma exertion trait into TMS rice plant become a promising solution to improve the RSUTMS.

Ideally, TMS plant requires a transgenic dominant gene for stigma exertion, so this trait can be an ideal function when it is introduced together with the dominant transgenic male sterility gene. In order to use recessive genes for the transgenic dominant gene, RNA interference technology or similar techniques are required. However there is a risk for unstable expression of this technology for a long-term application. Many scientists have been studied about stigma exertion and some QTLs for this trait already reported. The common and powerful QTL for stigma exertion was mapped, and the responsible gene for this QTL was cloned as *GS3* gene. *GS3* is a major gene for seed length, however the long and narrow grain and stigma exertion-type allele of *GS3* is recessive. Therefore, another QTL or gene which underlies stigma exertion must be searched for improvement of TMS.

As an initial step, QTLs for stigma exertion was searched within *O. sativa* used the F₂ population derived from crossing between 'Koshihikari' and 'CSSL3204'. Dr. Fukuoka constructed CSS lines using 'Naba' as donor trait for stigma exertion and

‘Koshihikari’ as genetic background, screened them, and found ‘CSSL3204’ has highest stigma exertion among the others. ‘Naba’ is *indica* rice from India has the high rate of stigma exertion among core collection that has been screened by Prof. Toriyama from Tohoku University. ‘Koshihikari’ is a leading *japonica* cultivar and frequently be used as parental lines in the breeding program in Japan because it possesses the best agronomic characteristics. One QTL region for the rate of exerted stigma (SER) was successfully detected on chromosome 2 on F₂ population through bulk segregant analysis (BSA); however, the dominance effect of this QTL is low. The result is also differing with the previous assumption that there was a putative QTL at ‘Naba’ region on the long arm of chromosome 3 which causes the ‘CSSL 3204’ has highest stigma exertion. Because the detected QTL derived from the first experiment is not suitable for the improvement of RSUTMS, another experiment was done using a donor from the wild rice.

Since the detected QTL in the first experiment was not suitable for improvement of TMS plant, screening of candidate donor material for dominant genes was expanded to the wild rice. F₂ population was used derived from crossing between *japonica* cultivar ‘Akidawara’ and *O. rufipogon* ‘W0120’. ‘Akidawara’ is one of the prospective cultivars in Japan while *O. rufipogon* was known as the common ancestor of rice. The ‘W0120’ should be preserved the dominant gene for stigma exertion trait based on its phenotypic appearance. Five QTLs for SER were detected in the F₂ population on chromosomes 2, 3, 4, 8, and 11. Except for the QTL on chromosome 4, the ‘W0120’ alleles increased SER. Two major QTLs, *i.e.*, *qSER3* was detected near the end of the short arm of chromosome 3, and *qSER8* was detected on the short arm of chromosome 8; had stronger dominance effects than the other QTLs and contributed to phenotypic variation were 14.5% and 12.6% respectively. The most commonly QTL for stigma exertion in rice (the QTL for *GS3*), was not detected in this population. A

validation using a BC₁F₁ population declared the two major QTLs which were detected using F₂ population has a clear dominant effect even though they have a low LOD value.

Core collection and wild rice preserve many useful traits for improvement of cultivated variety in the future, including for stigma exertion. Screening and selection for candidates of parental for target traits become important stage before introducing them into cultivated rice, to ensure the success of the goal. In the first experiment, the QTL region was detected on chromosome 2, however, because the donor trait derived from cultivated rice, this QTL has a low dominant effect and is not preferable for RSUTMS. In the second experiment, screening of donor trait from wild rice is effective to find out the QTLs which have high dominant effect for stigma exertion. Two detected QTLs with high dominant effect, *qSER3* and *qSER8*, have potential to be used for RSUTMS.

For recurrent selection in autogamous crop species, an efficient outcrossing system is required, because the flowering characteristics of rice are not suitable for efficient outcrossing. The dominant QTLs (*qSER3* and *qSER8*) may be used to improve the flowering habit and increase seed productivity in RSUTMS through cloning of the respective genes via fine mapping. Using one of the two genes may be sufficient to improve stigma exertion of TMS rice plant. Hopefully, detection of QTLs and application of the responsible genes that make autogamous crops become allogamous such as those detected in this study will improve RSUTMS and overcome the limitation in rice breeding.