The *Sub1* Gene and Its Implications in Developing Submergence-Tolerant Rice Cultivars

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Abstract

Submergence stress affects poor farmers living on 15 million hectares or more under rainfed lowlands in South and Southeast Asia. A major QTL on chromosome 9, *Sub1*, was previously mapped, and a cluster of three genes encoding putative ethylene responsive factors (ERF), i.e. *Sub1A*, *Sub1B*, and *Sub1C*, were identified at the *Sub1* locus. *Sub1* alone can provide tolerance to submergence in rice growing areas that are inundated by flash floods for up to two weeks. Using transformation, it was confirmed that *Sub1A* is the primary determinant of submergence tolerance. Recombinant plants within the *Sub1* cluster have also been identified recently, and preliminary data showed that *Sub1A* itself is enough to confer tolerance, while *Sub1C* by itself does not contribute to tolerance. In addition, the *Sub1* gene has been introduced into six widely grown varieties using a marker-assisted backcross approach. The first example of the submergence tolerant cultivars, Swarna-Sub1 has been successfully tested in the target regions, and field evaluation under control and submerged conditions for others are ongoing. In order to gain first insight of hybrid rice performance under submergence stress, the level of tolerance of heterozygous *Sub1A* tolerant allele is also being examined. An attempt has also been made to identify QTLs for submergence tolerance other than *Sub1*. Ultimately, the goal is to incorporate additional QTLs into Sub1-varieties to provide a higher level of tolerance under longer durations of submergence and for wider adaptation.

Media summary

Understanding how *Sub1A* and *Sub1C* interact with each other in conferring submergence tolerance and investigating whether the *Sub1* gene works in different genetic backgrounds

Key words

Sub1A, recombinant, marker-assisted backcross, submergence-tolerant, abiotic stress, *Oryza sativa*

Introduction

The *Sub1* QTL was first mapped in 1996 (Xu and Mackill 1996) and finally cloned and revealed as three ERF genes, named *Sub1A*, *Sub1B*, and *Sub1C* (Xu et al. 2006). It was also demonstrated that *Sub1A* is the main contributor for tolerance and that this gene could provide tolerance for up to two weeks of complete submergence. Recently this gene has been successfully introgressed through marker-assisted backcrossing (MAB) into a popular high-yielding variety from India, Swarna, within a 2-year time frame (Neeraja et al. 2007), which was equal to two rounds of backcrossing and one generation of self-pollination (BC_2F_2). This paper reports our work in developing six submergence-tolerant varieties using different mega varieties as recipients as well as confirming the role of *Sub1* in different genetic backgrounds. The important role of *Sub1A* and *Sub1C* will be further investigated, and the potential use of *Sub1* in hybrid rice will be examined.

Methods

The marker-assisted backcrossing (MAB) approach that we used for developing mega varieties carrying tolerance to submergence was described in Neeraja et al. (2007). Two prototypes derived from FR13A, IR49830-7-1-2-2 and IR40931-33-1-3-2 (referred as IR49830 and IR40931, respectively), were selected as donors. Besides Swarna, there were five other mega varieties used as recipient parents, namely Samba Mahsuri, IR64, Thadokkham 1 (TDK1), CR1009, and BR11. Populations used in searching for the recombinant plants within *Sub1* gene cluster were the same materials as the ones used for our MAB studies. Gene-based and intragenic markers developed within the *Sub1* region were used to identify the breakpoints within the recombinants. F_1 plants heterozygous for *Sub1* were derived from crosses between IR64 and IR64-Sub1. Submergence tests and expression studies for both recombinant and F_1 material were conducted as described by Xu et al (2006). New F_3 populations have been developed from several varieties identified as moderately tolerant and do not possess the *Sub1A-1* tolerant allele.

Results

Most of the tolerant versions of the mega varieties were produced within two rounds of backcrossing and one generation of self-pollination by MAB, as was done in Swarna-Sub1 (Neeraja et al, 2007). Only relatively small fragments coming from the donor at the top of chromosome 9, where the QTL *Sub1* is located, were introduced, and on average the size of the introgression was ~ 7Mb. In addition, improved versions of *Sub1* lines with smaller introgressions of the *Sub1* region could be recovered in the BC_3F_2 generation, and on average the size of the introgression was ~ 1.75 Mb. Evaluation of grain quality, yield performance, and agronomically important traits of the first-three *Sub1* lines at the IRRI farm (Swarna-Sub1, Samba Mahsuri-Sub1, and IR64-Sub1) was conducted under control and stress conditions. The *Sub1* lines performed superior under stress and there was no significant difference under non-stress conditions (data not shown). Upon completion of the conversion of the six mega varieties, evaluations of survival percentages of all six converted mega varieties showed that all the *Sub1* lines had significantly higher survival rate than the original parents (Figure 1).

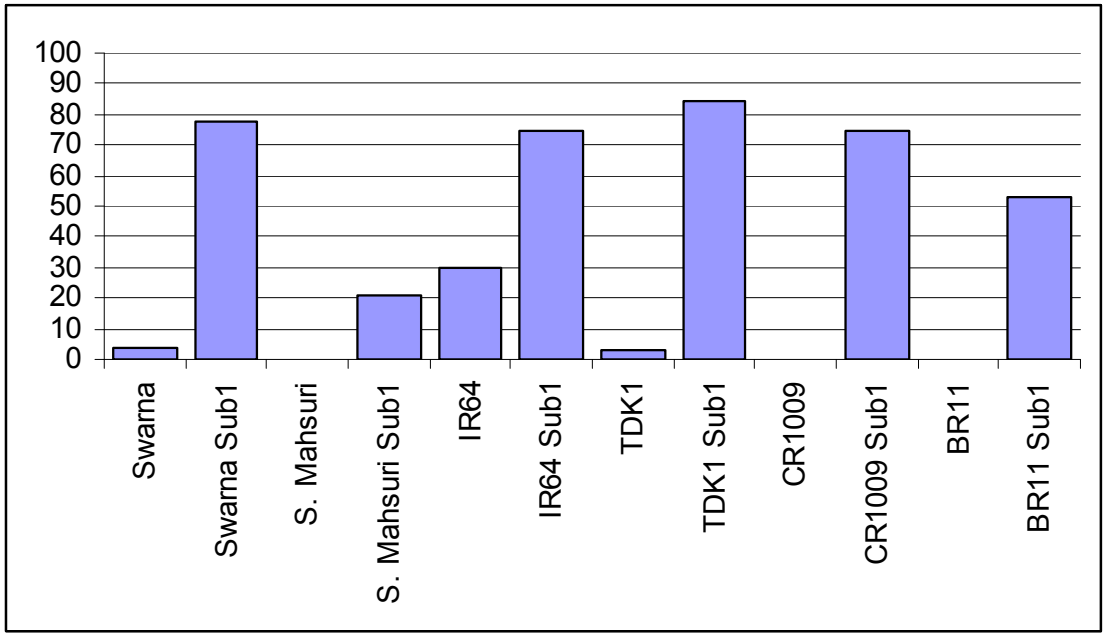

Figure 1. Submergence screening of the six *Sub1* lines showing percent survival of each converted line compared to its original mega-variety parent.

Using transformation, it was confirmed that *Sub1A* is the primary determinant of submergence tolerance (Xu et al, 2006). However, there has not been a clear understanding as to how *Sub1A* and *Sub1C* interact with each other in conferring the tolerance phenotype. In order to further elucidate this interaction, we searched for recombinant plants within the *Sub1* cluster using several populations segregating for *Sub1*. After screening

about five thousand plants in segregating populations, two recombinants between the *Sub1A* and *Sub1C* genes were identified. The two fixed recombinant lines, named "IR64-*Sub1BC*" (tolerant *Sub1B* and *Sub1C* alleles, intolerant *Sub1A* allele) and "BR11-*Sub1A*" (tolerant *Sub1A* allele intolerant *Sub1B* and *Sub1C* alleles) were used for submergence test and expression studies. Our submergence test data indicated that there was no significant difference in levels of tolerance among the IR64-Sub1BC lines and the recipient parent IR64. All of them showed an intolerant phenotype (Figure 2). Our data also showed that there were no significant differences in levels of tolerance among the BR11-Sub1A lines and the tolerant check BR11-Sub1. In this case, all of them showed the tolerant phenotype. Our expression studies showed that *Sub1A-1* down regulated the expression of the *Sub1C-3* intolerant allele (data not shown). These data confirmed the results reported by Xu et al., (2006). In addition, our results showed that the expression of *Sub1C-1* tolerant allele was independent of the *Sub1A* alleles, where in this case there was no accumulation of *Sub1C* mRNA. It was noted that limited expression of *Sub1C* was associated with tolerance (Xu et al., 2006). However, even though there was no expression of the *Sub1C-1* allele, in the presence of *Sub1A-2* the plants remained intolerant (data not shown)

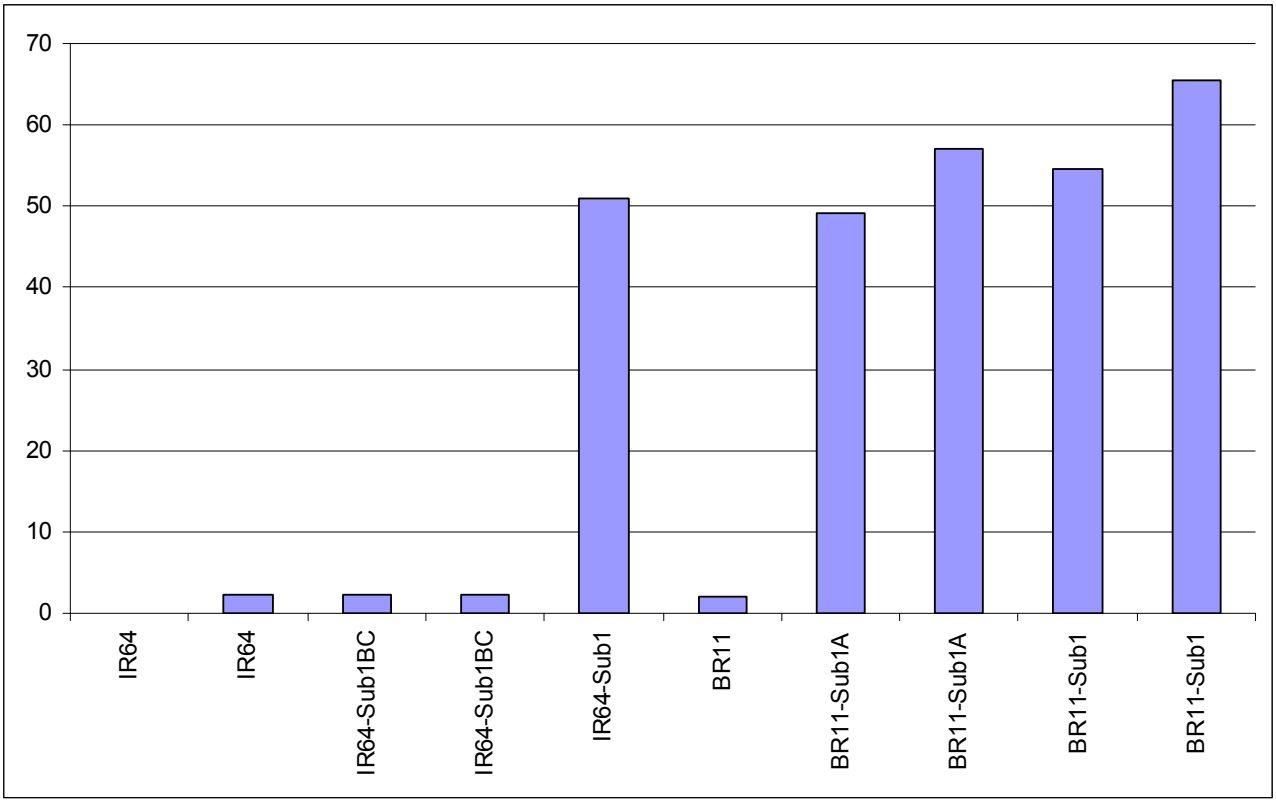

Figure 2. Submergence screening of the recombinants within *Sub1* cluster showing percent survival of each recombinant line compared to the original parents and *Sub1* lines.

With the increasing vulnerability of irrigated rice areas to flash floods, there is also a need to develop hybrid rice with submergence tolerance. In order to gain initial insight of the performance of a heterozygous *Sub1A-1* tolerant allele under submergence stress conditions, we measured the survival of F_1 hybrids of IR64/IR64-Sub1 in comparison to the parents. Our data indicated that the heterozygous plants were significantly less tolerant than the homozygous tolerant allele (Figure 3). In addition, the expression of *Sub1A* allele in heterozygotes is less than the tolerant ones (data not shown).

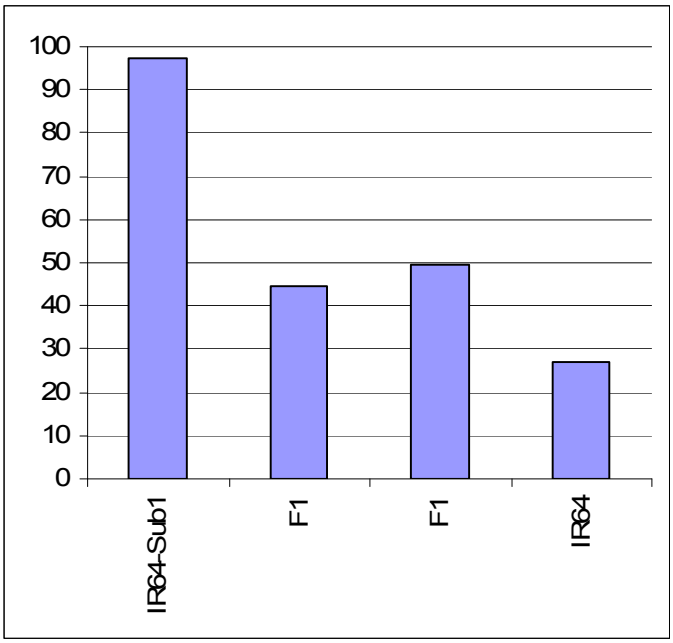

Figure 3. Submergence screening of the F_1 heterozygous *Sub1A* tolerant allele showing percent survival of the heterozygous F_1 individuals compared to the IR64 and IR64-Sub1 parents.

In order to identify additional submergence QTLs, we have identified several other promising donors that do not carry the same allele as FR13A. Screening for the F_3 populations are still ongoing. Genetic studies will be conducted for the promising populations.

Conclusion

The conversion of six mega varieties with submergence tolerance was successfully completed through MAB within a range of two to three years, and it was demonstrated that *Sub1*conferred tolerance in all genetic backgrounds used. Evaluation of grain quality, yield performance, and agronomically important traits of the *Sub1* lines conducted under control and stress conditions showed that the *Sub1* lines performed superior under stress and there was no significant difference under non-stress conditions. Our studies based on the recombinant plants identified within *Sub1* cluster confirmed our previous finding that *Sub1A* was the main contributor for tolerance. Furthermore, *Sub1C* by itself does not contribute to tolerance. Our results also showed that in order to maintain the high level of tolerance in a hybrid variety, both parents should carry the *Sub1A-1* tolerant allele.

References

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