Map-Based Cloning of the Quantitative Trait Locus *Seed Dormancy 10* in Rice (*Oryza Sativa L.*)

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Introduction

Seed dormancy (SD) is an adaptive trait of both ecological and agricultural importance and has been associated with multiple quantitative trait loci (QTL) in several crop or model plants. These QTL varied in effect on germination and are presumably underlain by genes differentiated during evolution. Cloning and characterization of the QTL cloud provide in-depth insights into evolutionary and developmental mechanisms of SD and also provide candidate genes to manipulate crop varieties for germination capability. We identified the QTL *Seed Dormancy 10* (*qSD10*) and introduced a *qSD10*-contraining chromosome segment from weedy into cultivated rice (Ye et al., 2010). This research aimed to clone *qSD10* and to characterize the QTL underlying gene for pleiotropic effects.

Major Results

qSD10 was associated with both seed dormancy and flowering time

An advanced backcross line, which has a *qSD10*-containing region of 10 cM from SS18-2 (weedy rice) in the EM93-1 (cultivated rice) genetic background, was used to identify recombinants. Eight recombinants with single sub-segments of the QTL peak-containing region were selected for progeny testing. Marker-trait association analysis for the progeny lines confirmed *qSD10*'s effect on SD (Figs. 1A & B) and revealed that *qSD10* also influences flowering time (Fig. 1D) and plant morphologies (data not shown). The allele of *qSD10* from SS18-2 increased SD and delayed flowering time.

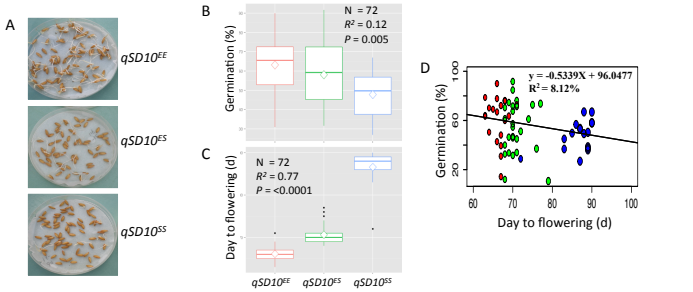

Fig. 1 Genotypic differences in seed dormancy and flowering time. **A.** Images showing difference in germination velocity. Genotypes are represented by the alleles from EM93-1 ($qSD10^{EE}$), SS18-2 ($qSD10^{SS}$), or both ($qSD10^{ES}$). **B & C.** Frequency distributions for % germination and days to flowering. **D.** Correlation between flowering time and % germination.

qSD10 was narrow to a genomic region containing a predicted Myb gene

New markers were developed to genotype the 8 recombinants to delimit *qSD10*. Marker-trait correlation analysis for seed dormancy revealed that *qSD10* locates between the markers InDel 4 and 27 (Fig. 2). This marker interval is ~170 Kb in physical length and encompasses the locus *Os10g32600* encoding a predicted Myb family transcription factor. This gene was reported to regulate flowering time (Doi et al., 2004). Thus, *Os10g32600* could be a candidate gene for *qSD10*.

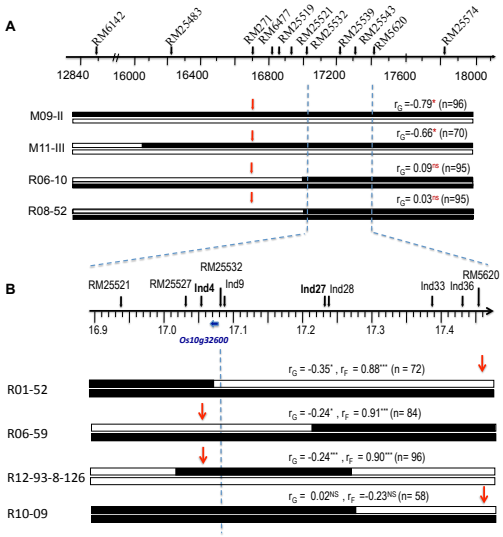

Fig.2 Fine mapping of *qSD10* with recombinants. **A.** Initial recombinants. The recombinants were identified by marker-assisted selection from the BC_1F_5 generation and evaluated for seed dormancy by progeny testing. **B.** Rare recombinants. The recombinants were identified from the BC_1F_6 generation and evaluated for seed dormancy and flowering time by progeny testing. Horizontal bars indicate chromosomal segments from the parental lines SS18-2 (dark) and/or EM93-1 (empty). Data shown are correlation coefficients (r) between marker (arrow-pointed) genotypes and germination percentages (r_G) or day to flowering (r_F) in the progeny line of n plants. The blue arrow below physical map is the predicted gene (*Os10g32600*) on the reference genome.

Sequence variation of a *qSD10* candidate gene between the EM93-1 and SS18-2 alleles

Genomic DNA and cDNA sequences for the *Os10g32600* locus were cloned from EM93-1 and SS18-2 rice to model a structure of the *qSD10* candidate gene (Fig. 3A). Sequence alignment also detected >20 point mutations (largely in introns) between the alleles from EM93-1 ($SD10^E$) and SS18-2 ($SD10^S$). Protein sequences deduced from the cDNAs consist of 341 amino acid (aa) residues and is annotated as an Myb family transcription factor (Fig 3A). Alignment of the protein sequences identified only one change at the 195th residue from Aspatic acid (D) in $SD10^E$ and Nipponbare ($SD10^N$) to Asparagine (N) in $SD10^S$.

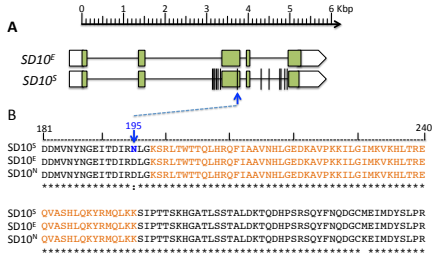

Fig.3 Allelic variation of the *qSD10* candidate gene *Os10g32600*. **A.** Gene structure. The structure was developed based on genomic DNA sequences from the parental lines EM93-1 ($SD10^E$) and SS18-2 ($SD10^S$). Boxes and line segments indicate exons (filled) or UTRs (empty) and introns, respectively. Vertical lines indicate point mutations at $SD10^S$. **B.** Protein sequences. The amino acid sequences were deduced based on cDNA sequences from EM93-1, SS18-2 and Nipponbare. The predicted H-T-H Myb domain is depicted by red color. The arrow indicates a change in amino acid residue.

Silencing the predicted Myb gene enhanced seed dormancy and delayed flowering time

An inverted repeat sequence (IRS) designed based on *Os10g32600*'s coding sequence was used to develop RNA interference (RNAi) lines in the Nipponbare background to confirm the gene function seed dormancy. Seed samples from five and one of the T1 and T2 RNAi generations and one BC1F1 generation shown different of DTF and degree of seed dormancy as evaluated by percentage germination. This results indicated that loss-of functional mutation of transcription factor (*Os10g32600*) not only result in enhanced degree of seed dormancy but delay flowering time in both Nipponbare genetic background (Fig. 4A and 4B) and EM93-1 genetic background background (Fig. 4D and 4E). To prove that enhanced seed dormancy and delay flowering time caused by loss-of-function of candidate gene, the transcription level of *Os10g32600* was quantified between wild type (Nipponbare and/or EM93-1) and RNAi lines (Fig. 4). The RNAi analysis demonstrated that the transcription level of target gene was decreased in RNAi lines compare to Nipponbare in T2 (Fig. 4C) and EM93-1 in BC1F1 (Fig. 4F) generation, respectively.

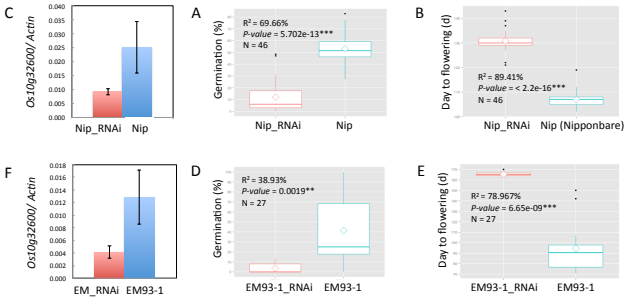

Fig. 4 Genotypic differences in the relative expression level of *Os10g32600*, seed dormancy and flowering time. Nip_RNAi is a transgenic T2 line with an RNAi structure to silence *Os10g32600* in the recipient Nipponbare (Nip). EM93-1_RNAi is a BC1F1 line with the RNAi structure in the EM93-1 background. Data shown are silencing effects in the Nipponbare (**A** to **C**) or EM93-1 (**D** to **F**) background.

Conclusion and Discussion

- *qSD10* was narrowed to a genomic segment of <170 Kb and the narrowed interval associated with both seed dormancy and flowering time. The allele from the weedy rice line SS18-2 enhanced seed dormancy and also delayed flowering.
- The Myb transcription factor gene at the *Os10g32600* locus is a *qSD10* candidate gene. Silencing the Myb gene enhanced seed dormancy and delayed flowering, indicating that the allele from SS18-2 is loss-of-functional.
- The effect of the narrowed *qSD10* on seed dormancy was also influenced by genetic backgrounds or environmental conditions. Research is being conducted to select more rare recombinants to reduce *qSD10*'s candidate gene pool and to characterize the Myb gene for molecular and cellular mechanisms regulating the development/release of seed dormancy.

References

Doi, K., Izawa T., Fuse T., Yamanouchi U., Kubo T., Shimatani Z., Yano M., Yoshimura A (2004) Ehd1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. Genes & Development 18 : 926-936.
Ye H, Foley ME, Gu X-Y (2010) New seed dormancy loci detected from weedy rice-derived advanced populations with major QTL alleles removed from the background. *Plant Science 179: 612-9.*

Acknowledgements

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