Characterization of a *Setaria viridis* mutant with late flowering under short-day conditions

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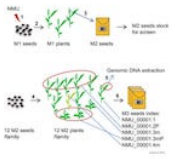
Background

➢ *Setaria viridis* as a model grass system

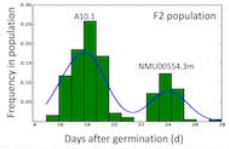
- Belongs to Panicoideae family, close to bioenergy crops (Miscanthus and switchgrass) and cereal crops (maize and sorghum).
- Small stature (10-20 cm tall).
- Short life span (6 weeks from seed to seed).
- Small genome size (~515Mb).
- C4 photosynthesis.

➢ Development and initial screening of NMU-mutagenized population (by Brutnell lab)

- The N-Nitroso-N-methylurea (NMU) mutagenesis generated 20,000 M2 families were generated.
- Approximately 2,700 M2 mutant families were initially screened, and one mutant (NMU00554.3m) was identified with delayed flowering and increased panicle length under short-day (SD) condition (12 h).
- The mutant was backcrossed to the parental line A10.1, the F2 plants displayed a segregation ratio 3:1 of delayed flowering and bigger panicle phenotype, and it inferred to be a recessive allele of a single gene.

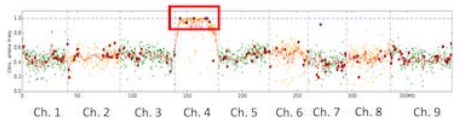


NMU mutagenesis and M2 screen protocol



The segregation pattern (3:1) in backcrossed F2

➢ Bulked-segregant analysis (BSA) by direct sequencing (by Brutnell lab)



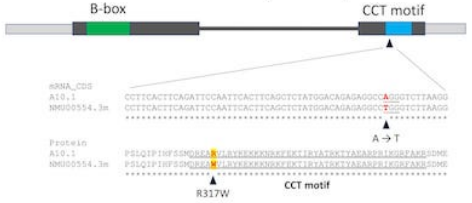
A 24 Mb wide QTL peak was detected in chromosome 4, it included 5 nonsynonymous single nucleotide polymorphisms (SNPs) within 4 candidate genes.

S. viridis gene ID	*A. thaliana* homolog ID	TAIR annotation
Sevir.4G059100	AT4G38180.1	FAR1-related sequence 5
Sevir.4G136300	AT5G53130.1	cyclic nucleotide gated channel 1
Sevir.4G187700	AT4G11000.1	Ankyrin repeat family protein
Sevir.4G194900	AT3G02380.1	CONSTANS-like 2

Results

➢ SvCO is homologues to Arabidopsis CONSTANS (CO) and rice Hd1

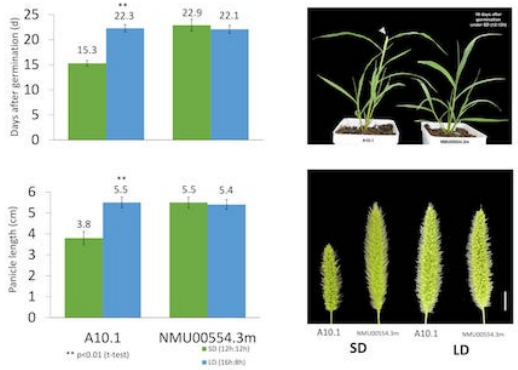
➢ A SNP located in the 2nd exon of *SvCO* caused an amino acid substitution (R317W)



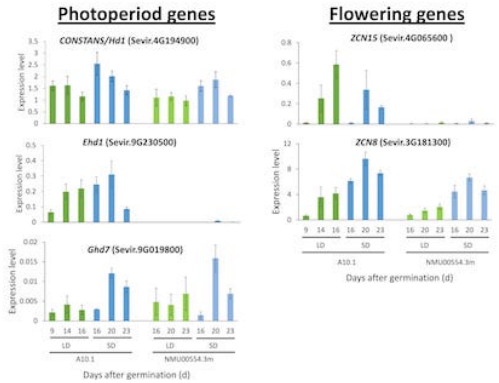
The SIFT analysis predicted that this R317W substitution was deleterious and would affect protein function.

➢ Mutational *SvCO* contributed the phenotypes

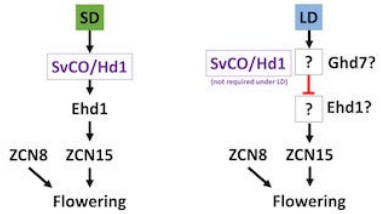
- The mutant only show late flowering and increased panicle length under SD condition, while the these phenotypes were not affected under long-day (LD) condition (16 h).



➢ Gene expression patterns revealed an atypical photoperiod signaling pathway in *Setaria* under LD



➢ Proposed photoperiod signaling models in *Setaria*



Future plans

➢ Examine the diurnal expression patterns of candidate genes.

➢ Protein-DNA binding assay to confirm SvCO activity.

➢ Yeast two-hybrid screening for identifying potential new signaling components.