**Methods**

The short reads from each sample were aligned to the *Setaria italica* reference genome (Sitalica\_164\_v2) using BWA [[1](#_ENREF_1)]. We used the single-end mapping mode of BWA with a maximal edit distance of 4% of the read length. For potential PCR duplicated reads, which were mapped to the same genomic position with same orientation, we only keep one read with the minimal edit distance. Furthermore, only reads that were uniquely mapped to the reference genome were kept. After mapping, a BAM file was generated for each sample by SAMtools [[2](#_ENREF_2)]. One mpileup file was then generated for the mutant line of interest by SAMtools, while a second mpileup file was also generated for the other mutant samples combined with the wile type which serve as a control. A base substitution or small indel (i.e. SNV), which is homozygous in the mutant line but absent from the control, was detected by comparing the two mpileup files. The SNV should be homozygous for the mutant line with an allele frequency > 85%, whereas the same allele is < 5% in the control. The substitutions were further filtered to keep only C:G → T:A transitions.

(The main text discussed how to prioritize potential causal mutations in detail. )

**Somewhere to be inserted in Results**

Although we used *Setaria italica* as the reference genome, about 84% of genome sequencing reads from *Setaria viridis* samples could be aligned to the genome. On the other hand, in doing so, we coerced the detected mutations to be resided in the genomic region conserved between the two *Setaria* species.

Zhangjun:  
  
Please see the following explanation (also in the attached Word). It is very detailed. Please select some descriptions, which you think is necessary, and add them to our method.

The library is PE sequencing, but I decided to use the single-end mapping mode to avoid the versatility of PE mapping. The read length is 100-bp, which is long enough to produce reliable mapping. If two reads were mapped to the same genomic position with same orientation, then these two reads were deemed as potential PCR duplicates. Only the read with the minimal edit distance was kept.

In identifying SNVs between the mutant line and control, we used 1 read coverage for either sample as the cutoff. We did not specify any mapping quality as long as the whole reads (no soft clipping) were uniquely mapped to the reference genome. The SNV should be homozygous for the mutant line with an allele frequency > 85%, whereas the same allele is < 5% in the control. Leila manually checked the list of SNVs and used certain criteria (see her main text) to select the most promising ones.

1. Li H, Durbin R: **Fast and accurate short read alignment with Burrows–Wheeler transform**. *Bioinformatics* 2009, **25**(14):1754-1760.

2. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, Subgroup GPDP: **The Sequence Alignment/Map format and SAMtools**. *Bioinformatics* 2009, **25**(16):2078-2079.