Progress Report 1 with figures

Objective 1: Develop efficient PAMless Cas9 and Prime Editing platforms for soybean.
This is a gene editing tool development objective that seeks to develop a Prime Editing system for making specific mutations in the soybean genome.

We previously reported promising data for successful prime editing in soybean based on preliminary tests in hairy roots. We were in the process of producing transgenic plants carrying five different versions of prime editing constructs. We now have multiple transgenic plants for each of these constructs that are now maturing in the greenhouse. As soon as seeds can be harvested, they will be planted, so that we can perform genotyping analyses to determine if the specified gene edits are inherited and if the transgene constructs were also inherited.

Objective 2: Apply base editing and Prime Editing to modify genes affecting soybean responses to drought.

No progress to report this period, because we are revisiting the prime editing approaches needed to carry out this objective.

Objective 3: Application of CRISPR-Cas-based gene editing to identify genes that are critical for SDS resistance in soybean.

In our last report, we presented data related to the screening of T_1 plants from eight events generated from two of the five constructs created in this project, DR1 and N1. For the DR1 construct, 121 T_1 plants were screened and none of them carried mutations in either of the two genes that were targeted. For the N1 construct, we screened 73 progeny plants for mutations in the eight genes that were targeted. We identified one heterozygous mutation in *Glyma.03G053500* carrying a deletion of five nucleotides in the protein coding sequence (Figure 1).

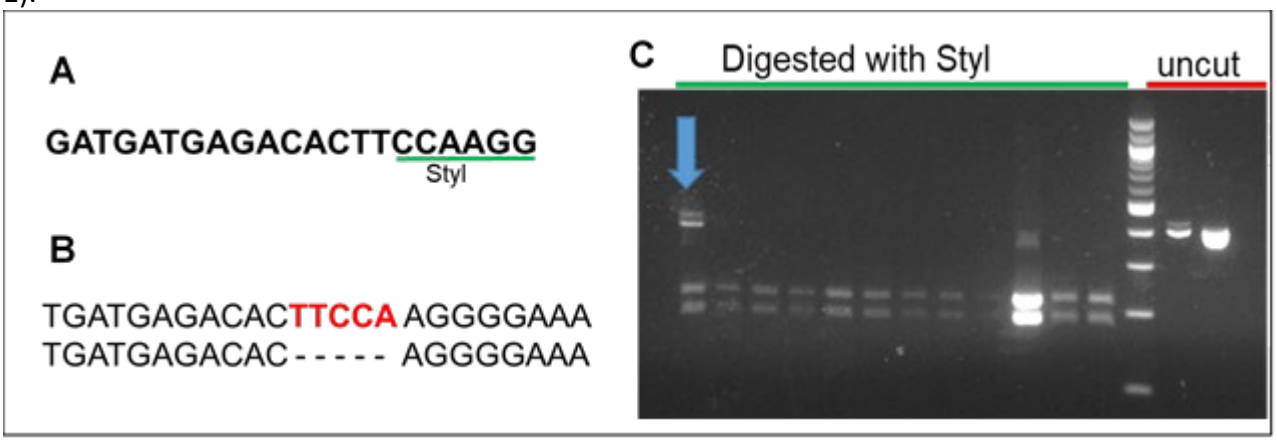


Figure 1. Restriction digestion analysis of the targeted sequence of *Glyma.03g053500*. A, gRNA with restriction site of StyI. B, Deletion of 5 base pairs identified from sequencing. C, Gel electrophoresis of the digested products. Only 1 plant out of 73 (blue arrow) was identified as a mutant.

We also reported that seeds were harvested from this heterozygote plant. As of today, we have analyzed the T_2 generation of the identified mutant. Out of 30 plants screened, 6 were homozygous for the mutation, 14 were heterozygous, and 10 possessed only the wild-type genotype (Figure 2). The numbers of homozygous mutants, heterozygotes and wild type progeny plants are consistent with the expected ratio indicating that the mutation was inherited as expected.

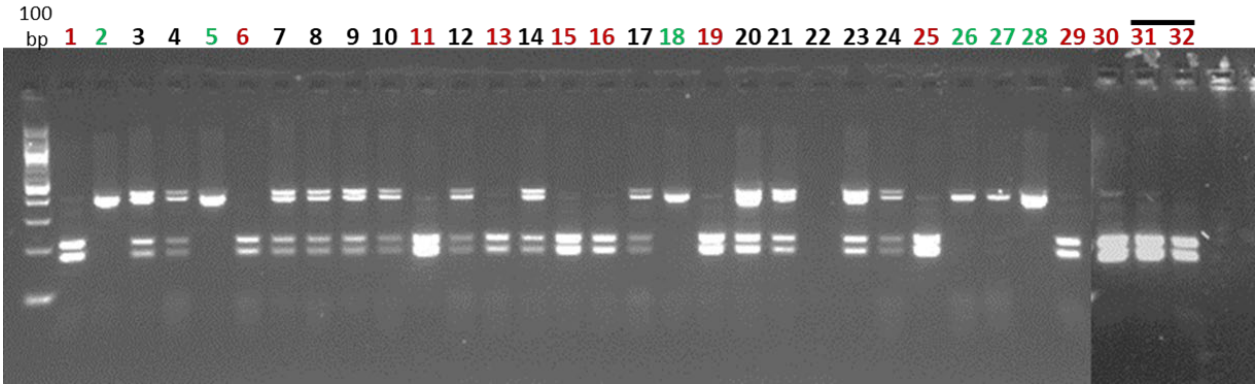
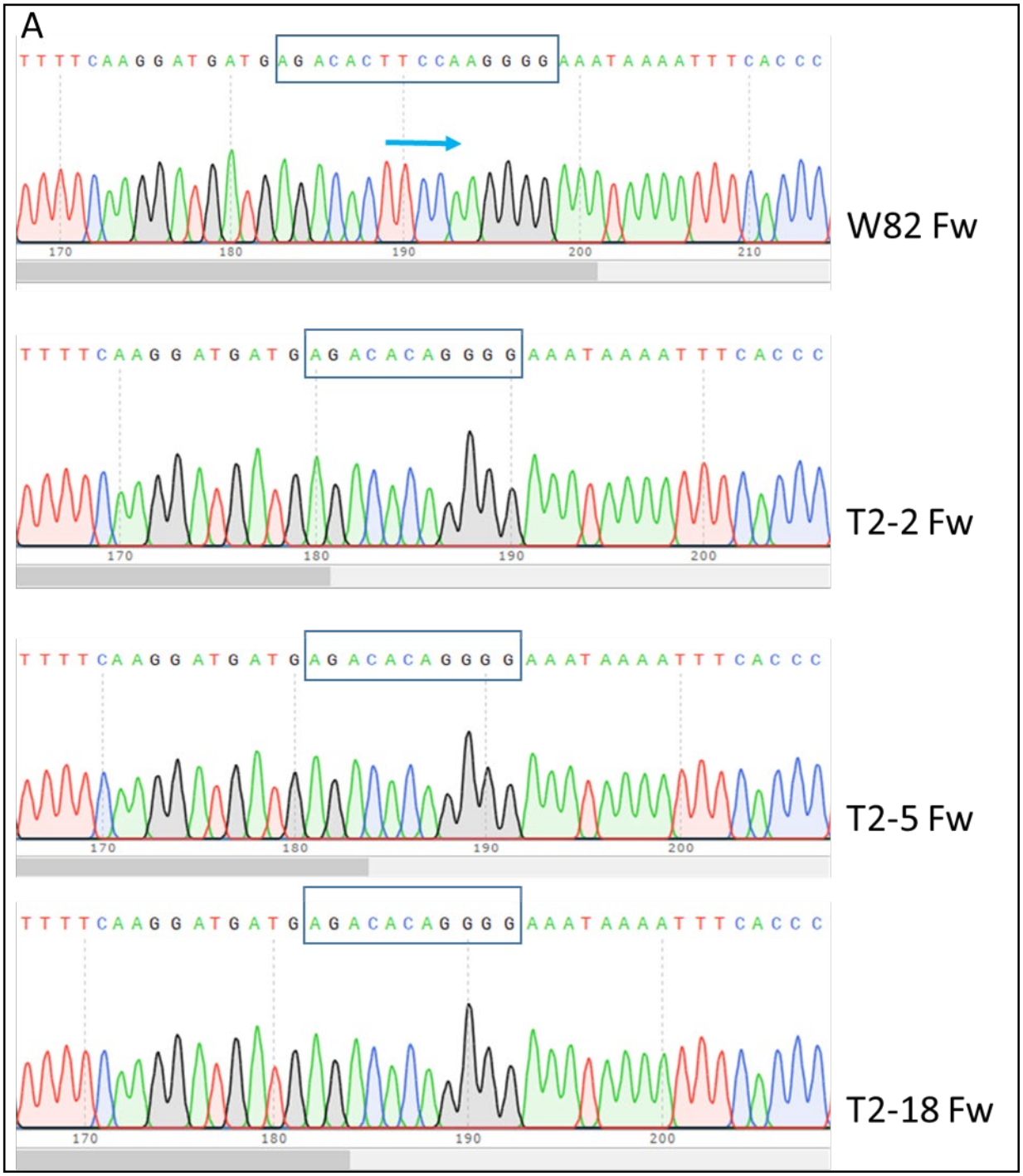


Figure 2. Restriction digestion analysis of the targeted sequence of *Glyma.03g053500* among the T_2 plants. Out of 30 plants analyzed, 6 are homozygote (green: 2, 5, 18, 26, 27, 28), 14 heterozygote (black), and 10 wild-type. The plant number 31 and 32 are wild type control (Williams 82).

We sequenced the PCR products from the six homozygous mutant progenies and the wild type. All six homozygous mutant progenies possessed the same deletion of the five nucleotides (Figure 3). These plants are being grown in the growth chamber. The seeds of these six homozygous plants will be grown to evaluate for the possible role of the disease resistance NLR protein encoded by *Glyma.03G053500* gene in disease resistance against *Fusarium virguliforme* and soybean cyst nematode.


A
W82 Fw
T2-2 Fw
T2-5 Fw
T2-18 Fw

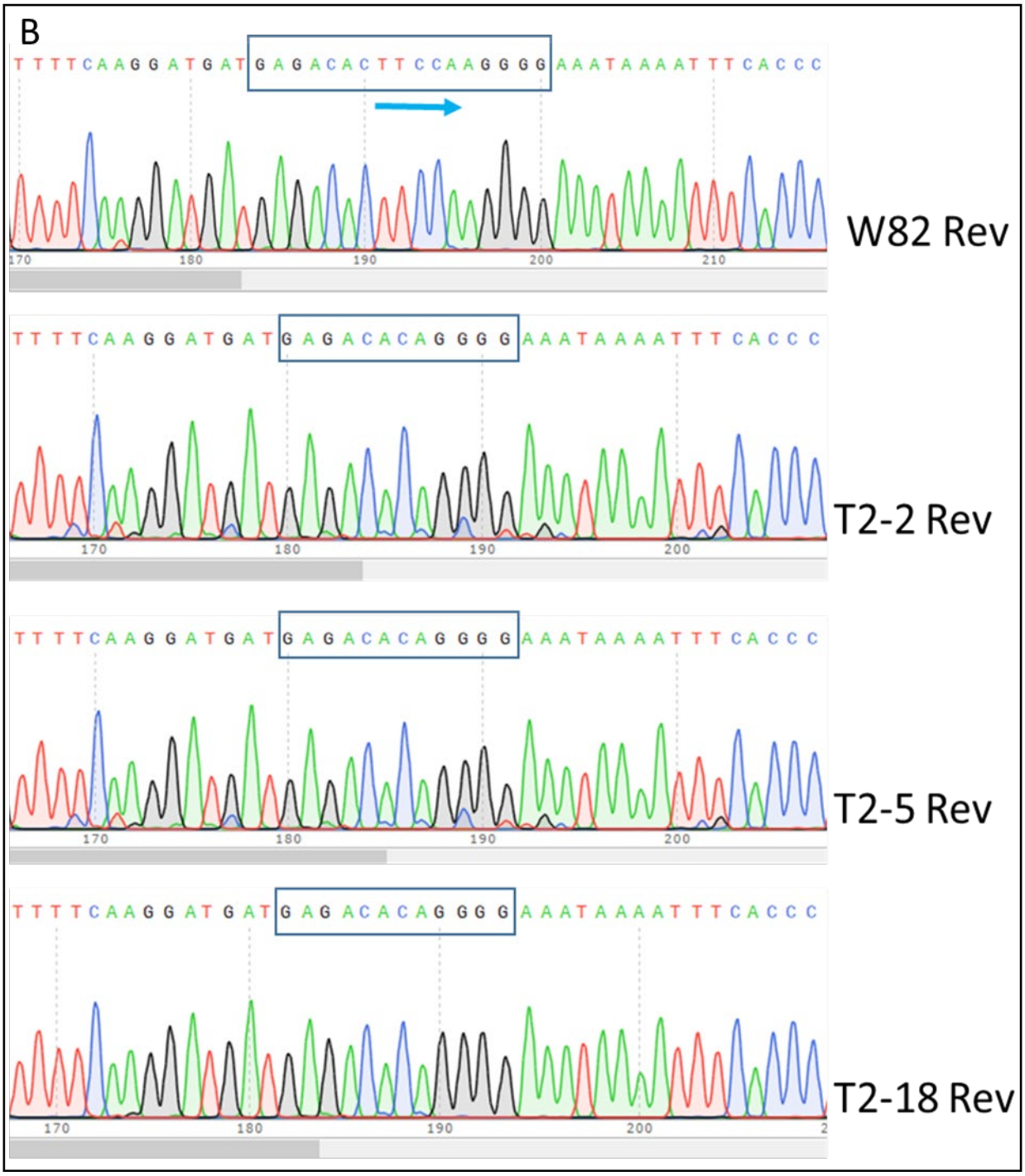


Figure 3. Sequencing pictograms of selected three mutants and wild type. A. Sequencing was conducted using the forward (Fw) primer. B. Sequencing was conducted using the reverse primer (Rev). Both strands of the mutant plants show the deletion of five nucleotides (shown by blue arrows in A and B).

All 30 plants were evaluated also for possible mutations in any of the other seven disease resistance genes, for which gRNAs were included in the construct for soybean transformation. However, no additional mutations were detected among these plants.