

Bowman-Birk inhibitor mutants of soybean generated by CRISPR-Cas9 reveal drastic reductions in trypsin and chymotrypsin inhibitor activities

Won-Seok Kim ¹, Jason D. Gillman ², Sunhyung Kim³, Junqi Liu ⁴, Madhusudhana R. Janga⁵, Robert M. Stupar⁶, Hari B. Krishnan ^{7*}

Supplementary Materials

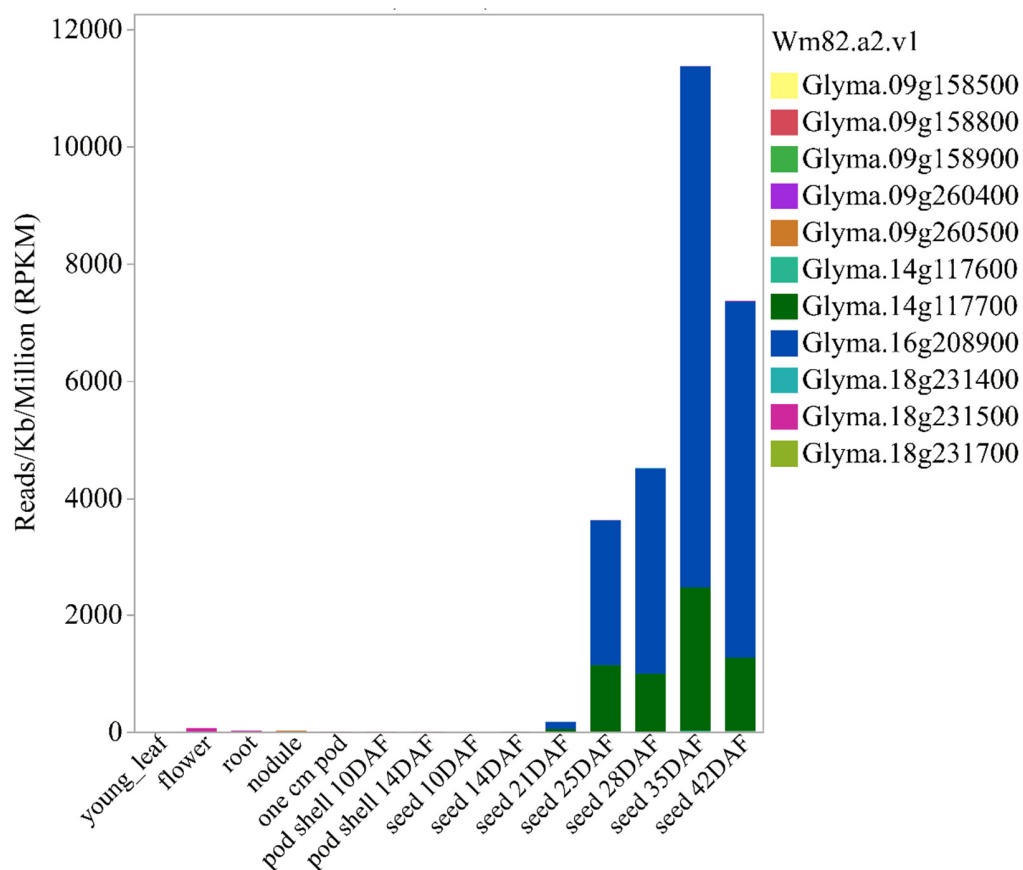


Figure S1. Gene expression of BBI genes in soybean. Data is repurposed from Severin et al. [20] using data housed on <https://soybase.org/soyseq/>. This RNAseq data was obtained from plant P-C609-45-2-2, a BC5F5 plant which was heterozygous for a seed protein QTL introgression from a *G. soja* (PI468916) into a *G. max* (A81-356022).

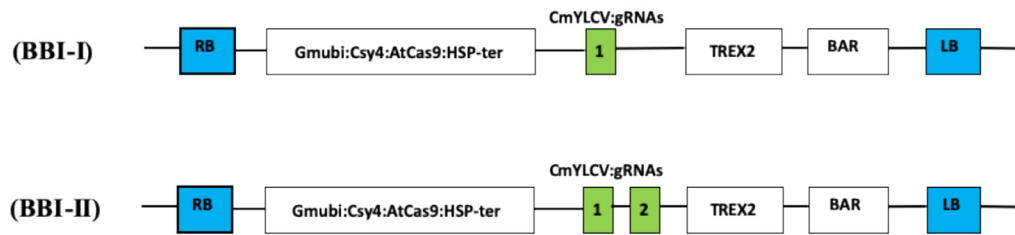


Figure S2. CRISPR-Cas9 cassettes targeting BBi genes. Events 5 was created using the BBI-II CRISPR-Cas9 construct and all other events were created through use of the BBI-I CRISPR-Cas9 construct.

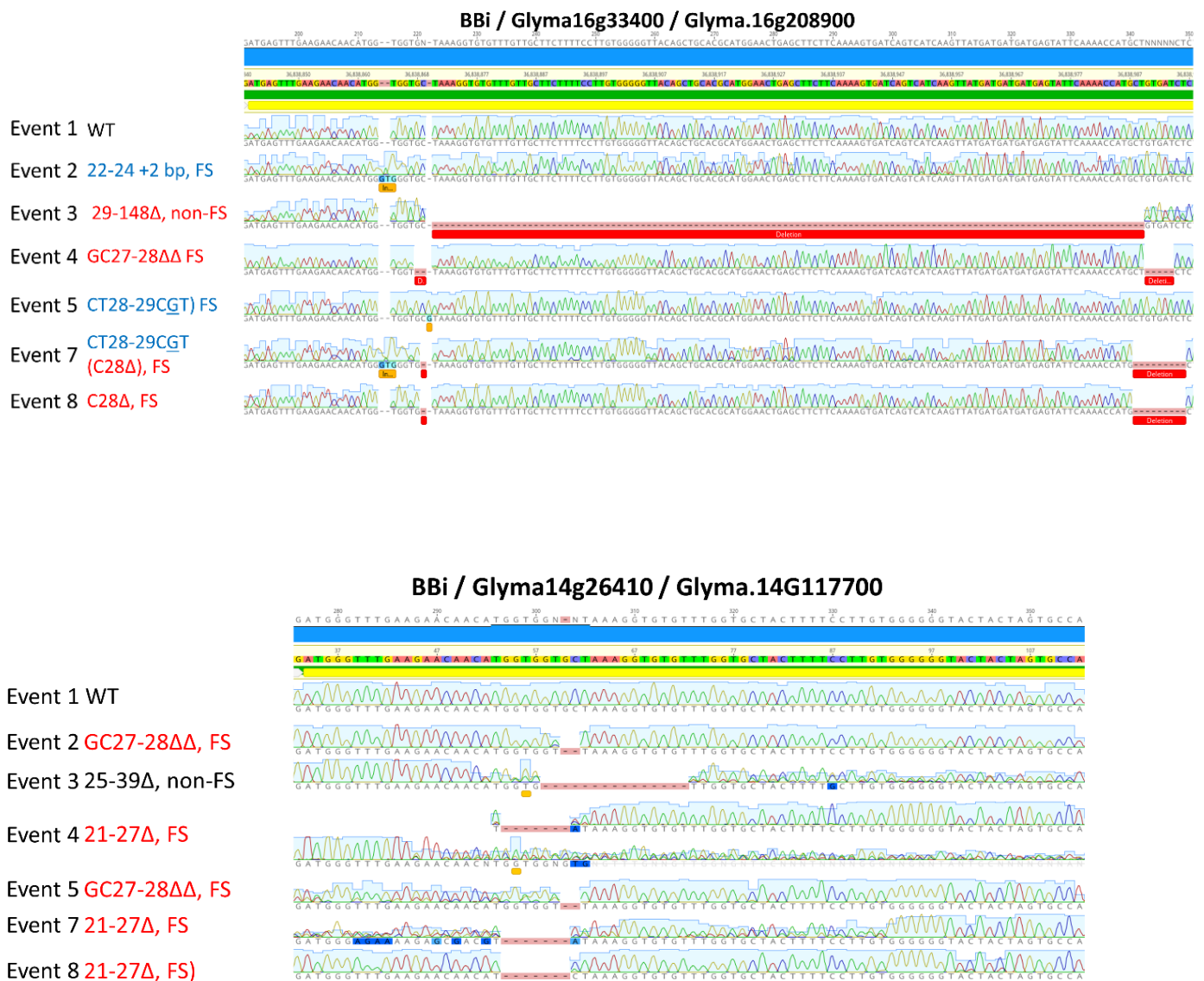


Figure S3. Sequencing traces for Glyma.16g208900 and Glyma.14G117700.

Table S1. Primers used for sequencing analysis and RT-PCR

Gene	Purpose	Primer name	Primer Sequence 5'- to -3'
Glyma.16g208900	PCR/sequencing	16G208900_WGF1	TAAATAACTCACTTCACGGGTTCT
Glyma.16g208900	PCR/sequencing	16G208900_WGR1	TGAACACACAAGTCGCATGA
Glyma.14G117700	PCR/sequencing	14G117700_WGF1	CAGTCATGATATCCTGCATGTGTG
Glyma.14G117700	PCR/sequencing	14G117700_WGR1	CCCATTGGAGAGAGAGCTTACAT
Glyma.16g208900	qRTPCR/sequencing	09G158500_WGF1	GAATTCGTGCCATTTCAGCTTGCAAATC
Glyma.16g208900	qRTPCR/sequencing	09G158500_WGR1	AACAAAGCATAAAGGGGGCTTTGTCCG
Glyma.14G117700	qRTPCR/sequencing	14F117700_WGF1	GAATTCGTGCCATTTCAGCTTGCAAATC
Glyma.16g208900	qRTPCR/sequencing	14F117700_WGR1	ACAAAGCCTAAAGGGGGCTTTGCCCA
Glyma.16g208900	qRTPCR/sequencing	09G158600_WGF1	AATTCATGTCACTCAGCTTGTGATCGC
Glyma.14G117700	qRTPCR/sequencing	09G158600_WGF1	CCTAAAGGGCTTTGTTCATTTGAGAGAG
Glyma.16g208900	qRTPCR/sequencing	09G158700_WGF1	AATTCATGTCACTCAGCTTGTGATCGC
Glyma.16g208900	qRTPCR/sequencing	09G158700_WGR1	CCTAAAGGGCTTTGTTCATTTGAGAGAA
Glyma.14G117700	qRTPCR/sequencing	16G208900_WGF1	AGCTTCTTCAAAAGTGATCAGTCATCAAGTTATG
Glyma.16g208900	qRTPCR/sequencing	16G208900_WGR1	GGCTGTGAGCGTGTGCACATACAGCTC