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

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**Supplementary Materials**

**Chart

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**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).

Diagram, waterfall chart

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**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.

**Graphical user interface

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**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 | GAATTCGTGCCATTCAGCTTGCAAATC |
| Glyma.16g208900 | qRTPCR/sequencing | 09G158500\_WGR1 | AACAAAGCATAAAGGGGGCTTTGTCCG |
| Glyma.14G117700 | qRTPCR/sequencing | 14F117700\_WGF1 | GAATTCGTGCCATTCAGCTTGCAAATC |
| 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 |