

Identification of Loci and Candidate Genes Responsible for Pod Dehiscence in Soybean via Genome-wide Association Analysis across Multiple Environments

Running title: GWAS for pod dehiscence in soybean

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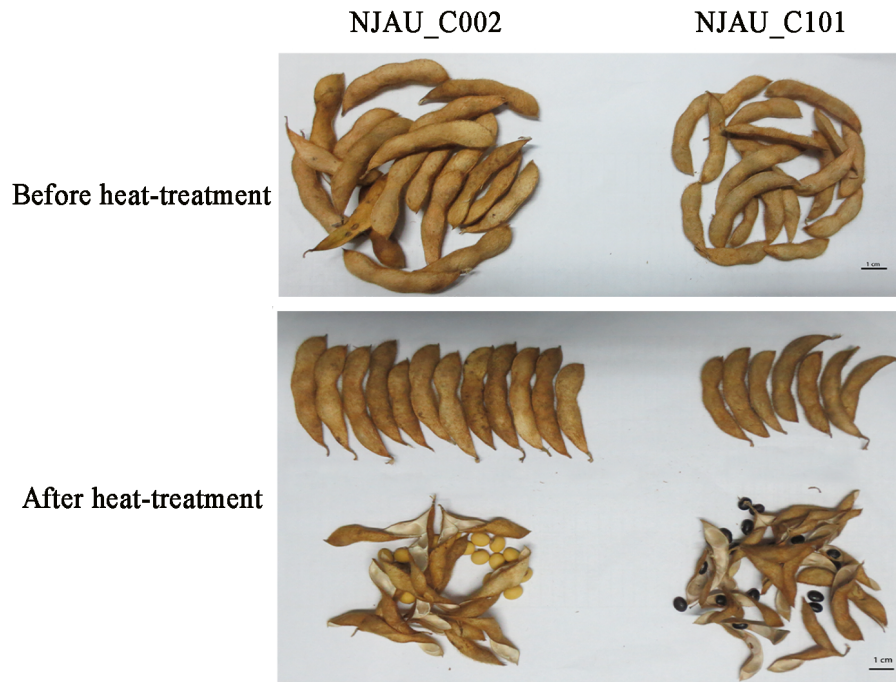


Figure S1. The phenotype of pod dehiscence between two soybean accessions (NJAU_C002 and NJAU_C101) before and after heat-treatment.

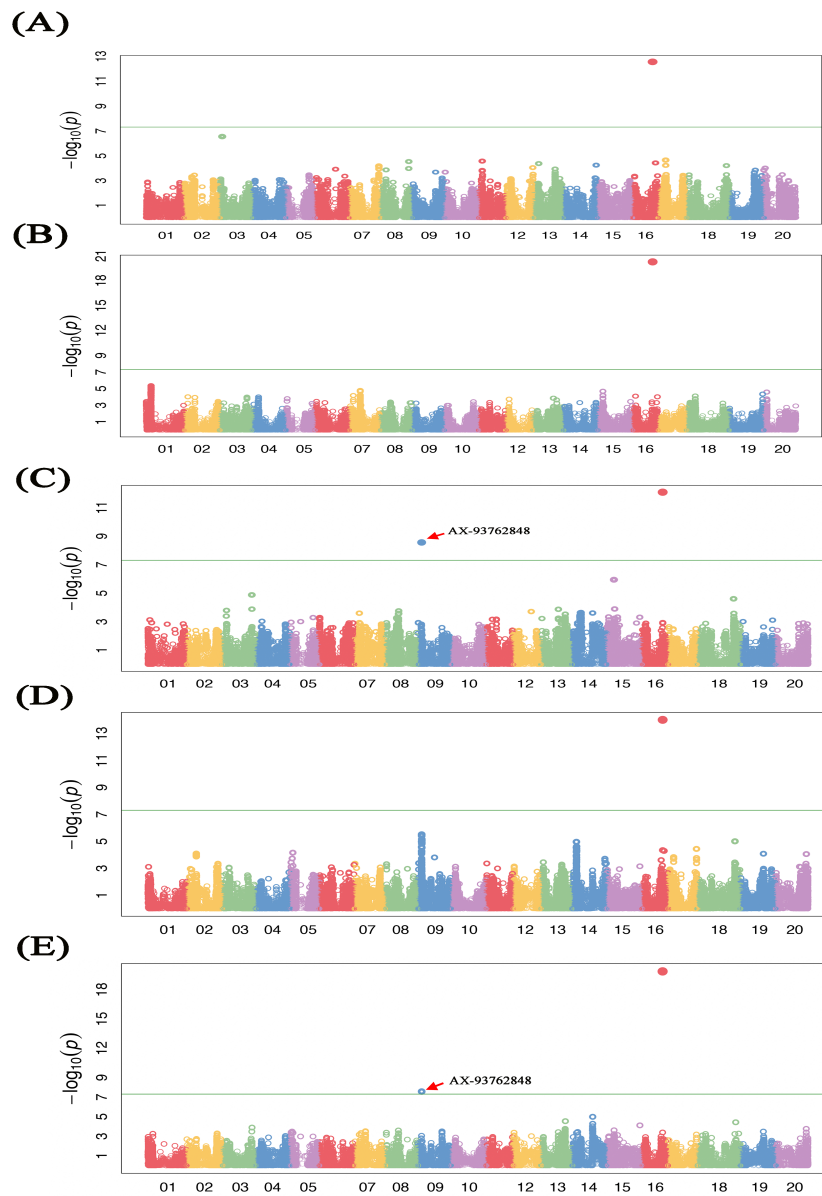
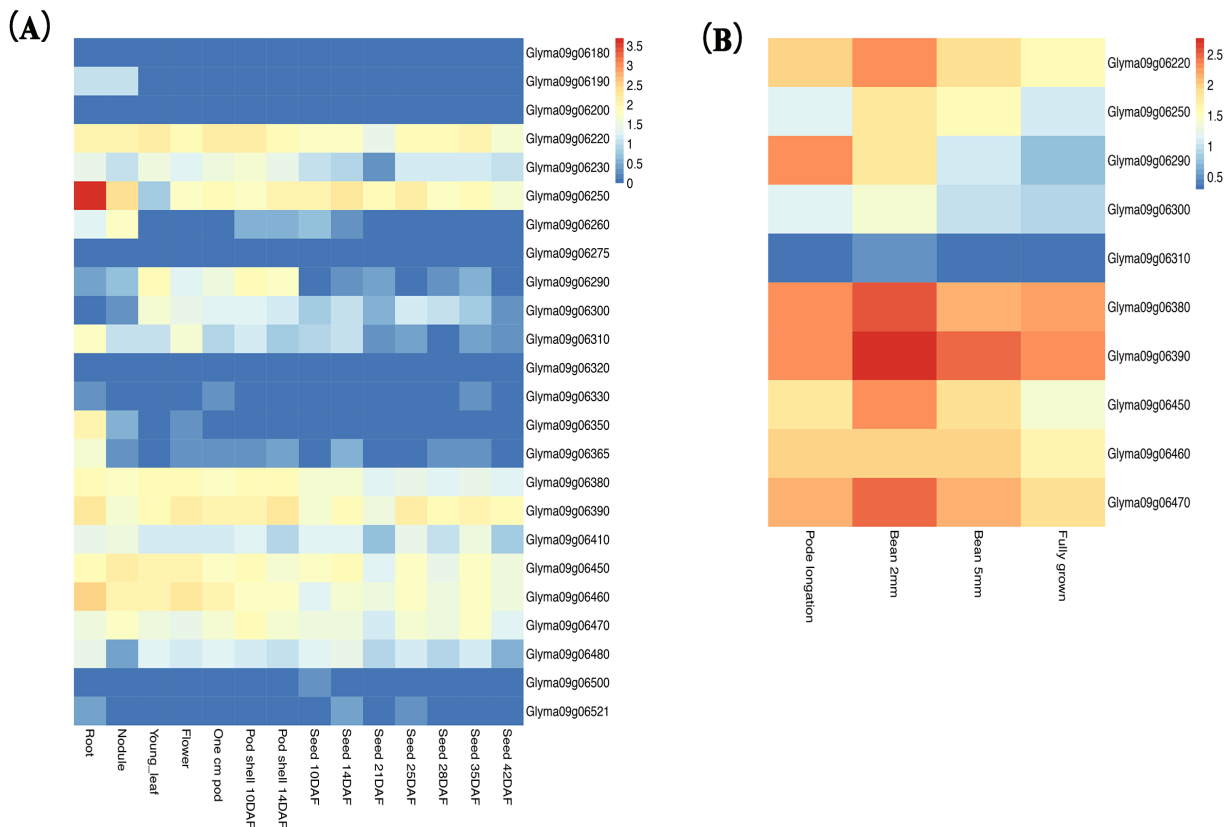


Figure S2. The Manhattan plots for pod dehiscence using MMLM model in the following environments: (A) Env1; (B) Env2; (C) Env3; (D) Env4; (E) Env5. The SNP indicated by the arrow is AX-93762848.



Supplementary Figure S3. Digital expression profiles for 26 genes in various tissues. (A) Expression levels of 26 genes in three vegetative tissues and at different seed developmental stages based on RNA sequencing data. The reads per kilobase million-normalized values were \log_{10} -transformed. (B) Expression of 26 genes in four different pod development stages from microarray data. The RMA-normalized values from the microarray data were \log_{10} -transformed. The transformed values were employed to create the heat map using R software.

Hap1 ¹ TGTTGTACGTGCCTGTGCACACTGACTCTCCCTATAATCACTCTCCACAGAAAACCGTTTTCTAGGCCTGTGGTCTTCTTTTTGTGCGTGTGCGTGCCTCCATACTTGCTTCGCCTAGTAATTATTATAATCCAGTT
Hap2 TGTTGTACGTGGCTGTGCACACTGACTCTCCCTATAATCACTCTCCACAGAAAACCGTTTTCTAGGCCTGTGGTCTTCTTTTTGTGCGTGTGCGTGCCTCCATACTTGCTTCGCCTAGTAATTATTATAATCCAGTT

TCCTTATCACTCCAATTATTCAATTACATGGGGCCTCCTCATGTCAAGGAAAAAATAAAAATTTTATTTGCTAGTGAACCTCACTTCCCTATAACTTGAACATATCTTCAACACTAACTTCTCCTCAACCCACACCATT
 TCCTTATCACTCCAATTATTCAATTACATGGGGCCTCCTCATGTCAAGGAAAAAATAAAAATTTTATTTGCTAGTGAACCTCACTTCCCTATAACTTGAACATATCTTCAACACTAACTTCTCCTCAACCCACACCATT

² CCCCC-TAAAAGCTTGTTTTTGCCTTCTTCTTCTCCTCAACATAGCTGACAAAAACAACCACTTCCCTCTTTCATTGTTCTCTCCCCCACTTTCACTATACCCCCCTTGCTTTACTTTGCATATTTTGCACCCTTT
 CCCCC-TAAAAGCTTGTTTTTGCCTTCTTCTTCTCCTCAACATAGCTGACAAAAACAACCACTTCCCTCTTTCATTGTTCTCTCCCCCACTTTCACTATACCCCCATTGCTTTACTTTGCATATTTTGCACCCTTT

³ TAACTTCTATTATCACTCACAATTCTGTTCTTGGTCTTCTTCTTTCTCATCCTTATACACTTCTTCTTAGATTGTTCTTGGTGGATTATTCATGGAGGGTCAACCAGCTAAAAGGAGACGTGTATACTCACT
 TAACTTCTATTATCACTCACAATTCTGTTCTTGGTCTTCTTCTTTCTCATCCTTATACACTTCTTCTTAGATTGTTCTTGGTGGATTATTCATGGAGGGTCAACCAGCTAAAAGGAGACGTGTATACTCACT

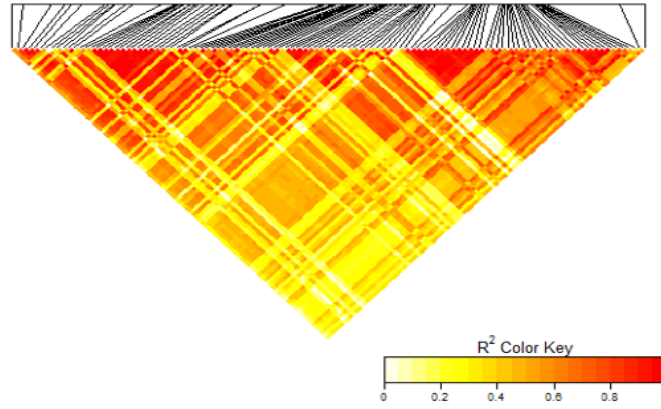
^{633bp} -----TTACTATTATGTATGTGTTCCACATATAATATATATTGTCAATTATCAAAGCAACTATATATTTATATAGTATGCTGTACTCTTTTCTTTTTATTTTAAATATCGTGTATATATTTTCAAATCTGTT
 -----TTACTATTATGTATGTGTTCCACATATAATATATATTGTCAATTATCAAAGCAACTATATATTTATATAGTATGCTGTACTCTTTTCTTTG-ATTTTAAATATCGTGTATATATTTTCAAATCTGTT

⁴⁵

Supplementary Figure S4. Comparison of sequence between Hap1 and Hap2. 1-5 represents S₋₅₀₀, Indel₋₂₃₀, S₋₁₂₈, Indel₋₇₆₆ and S₋₇₆₇. *: the translation start codon.**

Pairwise LD in r^2

Physical Length: 725kb



Supplementary Figure S5. LD analysis of the 136 associated SNPs on chromosome 16.

Supplemental Table S2. Primer sequence of qRT-PCR

Gene	Primer	Primer sequence and probe sequence
<i>Glyma09g06290</i>	Forward primer	AGAAGATGATGATGGTGGTGGTGAT
	Reverse primer	TTGTAGCTCAGTCACCATTTGTTC
<i>Glyma09g06320</i>	Forward primer	GCTCACGAAGGGAGTGAGAT
	Reverse primer	TGACGTGATTCAGAGTACACAC
<i>Glyma09g06390</i>	Forward primer	ATGAAGGCAATGAACCAA
	Reverse primer	TGCGGAATAATGCAACACC
<i>Tubulin</i>	Forward primer	CACTTACGCATCACATAGCA
	Reverse primer	GGAGTTCACAGAGGCAGAG