Genomics-assisted prediction of salt and alkali tolerances and functional marker development in apple rootstocks

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Abstract

Background: Saline, alkaline, and saline-alkaline stress severely affect plant growth and development. The tolerance of plants to these stressors has long been important breeding objectives, especially for woody perennials like apple. The aims of this study were to identify quantitative trait loci (QTLs) and to develop genomics-assisted prediction models for salt, alkali, and salt-alkali tolerance in apple rootstock. Results: A total of 3,258 hybrids derived from the apple rootstock cultivars ‘Baleng Crab’ (Malus robusta Rehd., tolerant) × ‘M9’ (M. pumila Mill., sensitive) were used to identify 17, 13, and two QTLs for injury indices of salt, alkali, and salt–alkali stress via bulked segregant analysis. The genotype effects of single nucleotide polymorphism (SNP) markers designed on candidate genes in each QTL interval were estimated. The genomic predicted value of an individual hybrid was calculated by adding the sum of all marker genotype effects to the mean phenotype value of the population. The prediction accuracy was 0.6569, 0.6695, and 0.5834 for injury indices of salt, alkali, and salt–alkali stress, respectively. SNP182G on MdRGLG3 , which changes a leucine to an arginine at the vWFA-domain, conferred tolerance to salt, alkali, and salt-alkali stress. SNP761A on MdKCAB , affecting the Kv_beta domain that cooperated with the linked allelic variation SNP11, contributed to salt, alkali, and salt–alkali tolerance in apple rootstock. Conclusions: The genomics-assisted prediction models can potentially be used in breeding saline, alkaline, and saline-alkaline tolerant apple rootstocks. The QTLs and the functional markers may provide insight for future studies into the genetic variation of plant abiotic stress tolerance.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

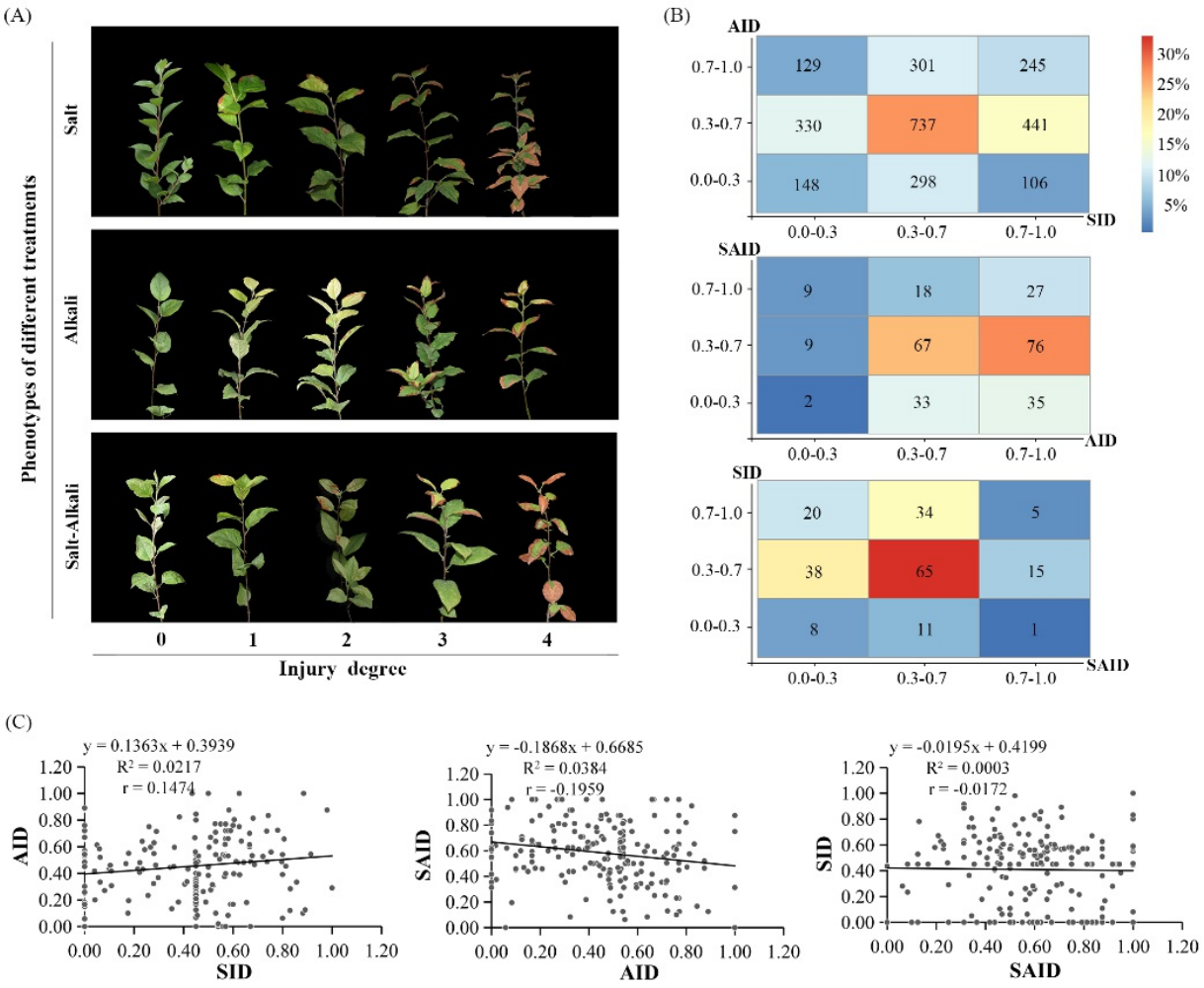

Figure 1

Categorical criteria of injury indices and pairwise relationship between injury indices of salt (SID), alkali (AID), and salt-alkali (SAID) stress in apple rootstock hybrids of Malus robusta Rehd. ‘Baleng Crab’ × M. pumila Mill. ‘M9’. (A) Photograph showing scoring criteria of injury degrees of salt, alkali, and salt–alkali stress. (B) Heatmap showing the pairwise relationship between numbers of hybrids tolerant or sensitive to salt, alkali, and salt–alkali stress. The number of hybrids are presented in the blocks and the percentage of hybrids by column is illustrated by color gradients. (C) Linear regressions between SID, AID, and SAID.

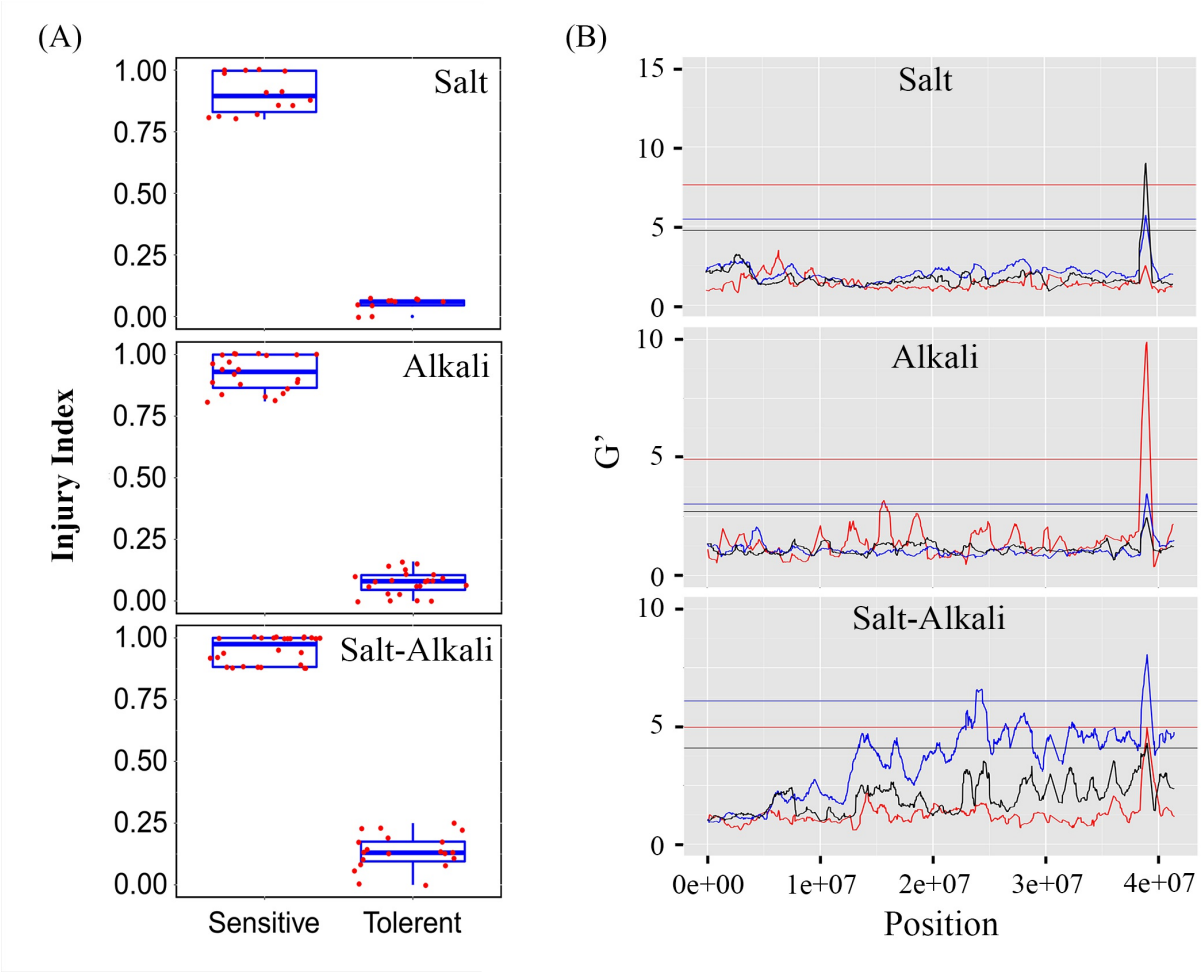

Figure 2

Diagrams showing phenotypes of the six segregant bulks with phenotype extremes for tolerant or sensitive hybrids (A) and common QTLs on chromosome 16 (B) for salt, alkali, and salt–alkali injury indices identified using bulked segregant analysis by sequencing F1 hybrids of Malus robusta Rehd. 'Baleng Crab (BC)' × M. pumila Mill. 'M9' rootstocks. In panel B, the Y-axis represents the G' value and the X-axis represents the physical position on the chromosome. The red curved lines: 'M9'; blue curved lines: 'BC'; black curved lines: 'BC' & 'M9'. The colored horizontal lines indicate the corresponding statistically significant threshold of the G' value.

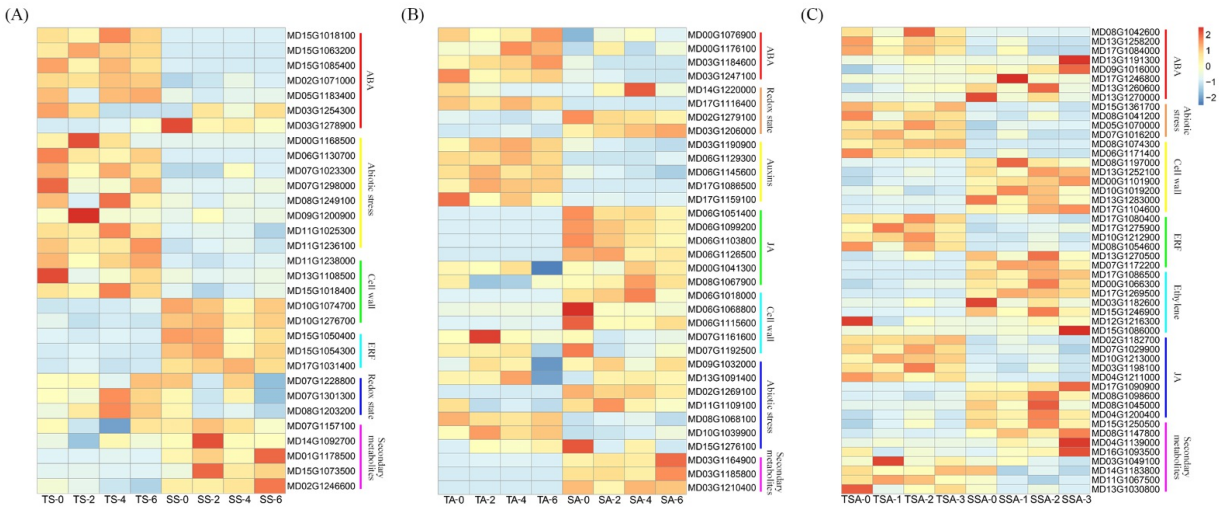

Figure 3

Heatmaps showing differentially expressed genes between individuals that were tolerant or sensitive to salt (A), alkali (B), and salt–alkali stress (C) in F1 hybrids of Malus robusta ‘Baleng Crab’ × M. pumila ‘M9’ rootstocks.

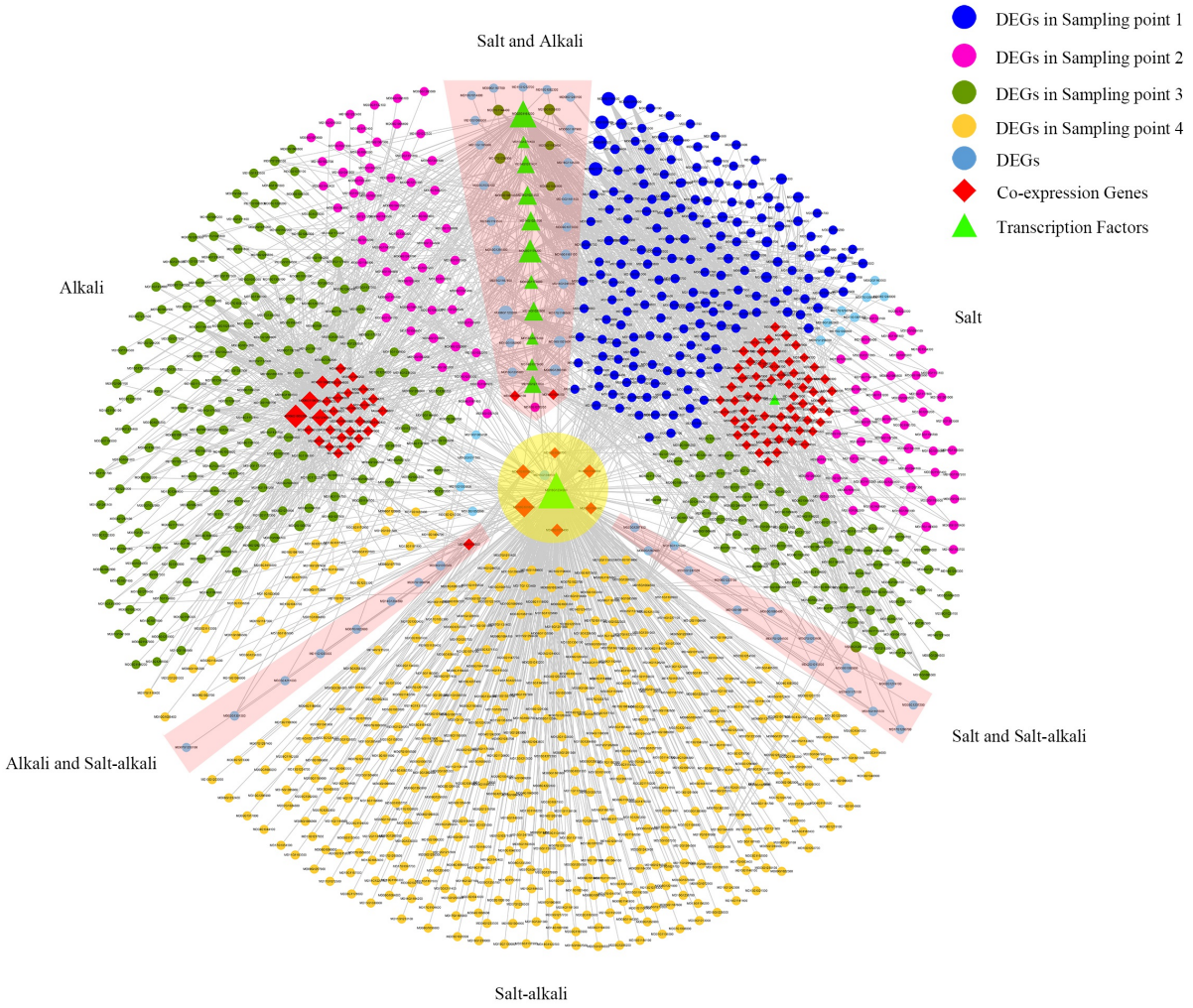

Figure 4

Co-expressions network of differentially expressed genes (DEGs) between individuals that were tolerant or sensitive to of salt, alkali, and saltâ€“alkali stress conditions in F1 hybrids of Malus robusta â€˜Baleng Crabâ€™ Ã— M. pumila ‘M9’ rootstocks.

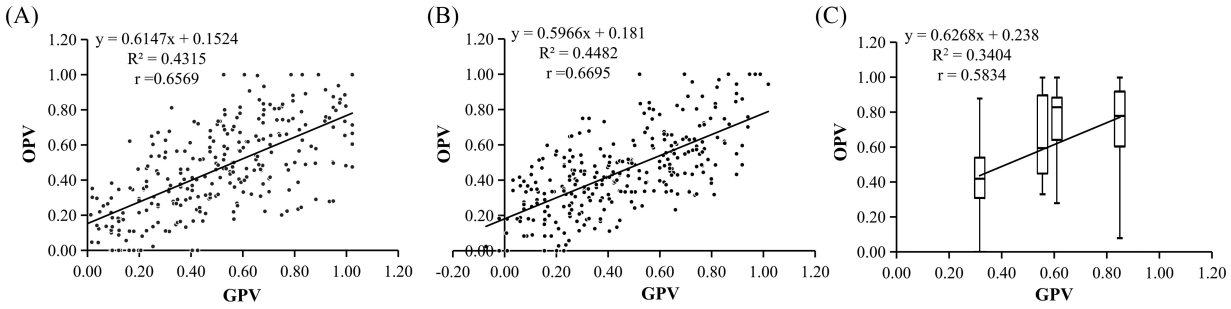

Figure 5

Linear regression between genomics predicted value (GPV) and observe phenotype value (OPV) of injury indices of salt (A), alkali (B), and saltâ€“alkali stress (C) in F1 hybrids of Malus robusta â€˜Baleng Crabâ€™ Ã— M. pumila â€˜M9â€™ rootstocks.

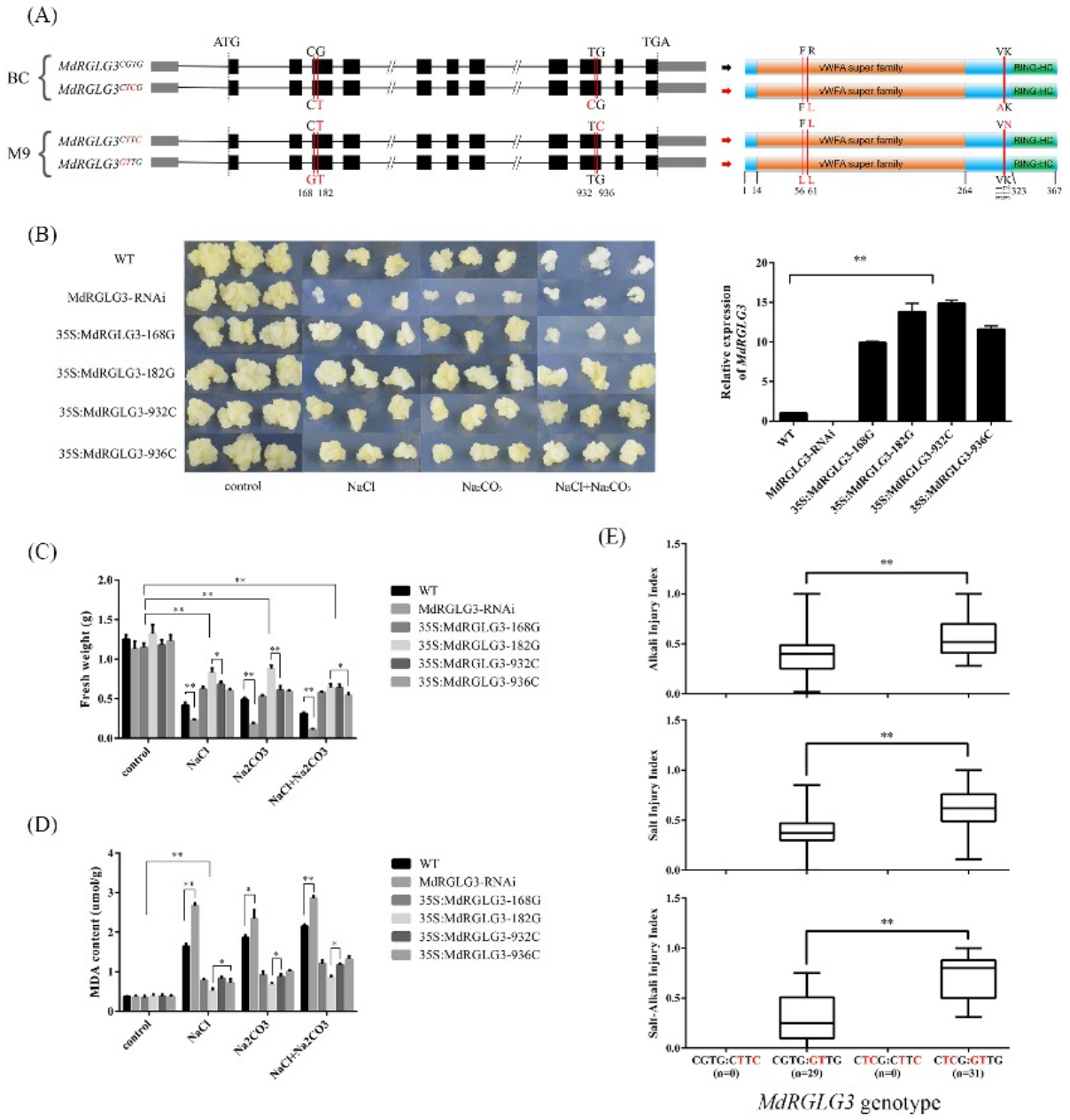

Figure 6

Schematic showing genetic variations on the coding sequence of MdRGLG3 and functional validation of these variations via callus transgenesis and genotype–phenotype association. A: Schematic diagram showing single nucleotide polymorphisms on the coding region and variations in the amino acid sequences of MdRGLG3. B: Images showing in vitro growth of transformed apple callus overexpressing variants of MdRGLG3 and the MdRGLG3-RNAi line on media containing high levels of NaCl, Na2CO3, or NaCl + Na2CO3. C and D: Column blots showing differences in apple callus fresh weight (C) and malondialdehyde (MDA) content (D)

14 days after treatment with NaCl, Na2CO3, or NaCl + Na2CO3, among transformants overexpressing variants of MdRGLG3 or the MdRGLG3-RNAi line. E: Box-plot showing injury indices of salt, alkali, and salt–alkali stress in F1 hybrids of ‘Baleng Crab’ × ‘M9’ with different genotype combinations of MdRGLG3 SNP168, SNP182, SNP932, and SNP936. Data are means ± standard deviation of three biological replicates. *, **, and *** indicate statistically significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

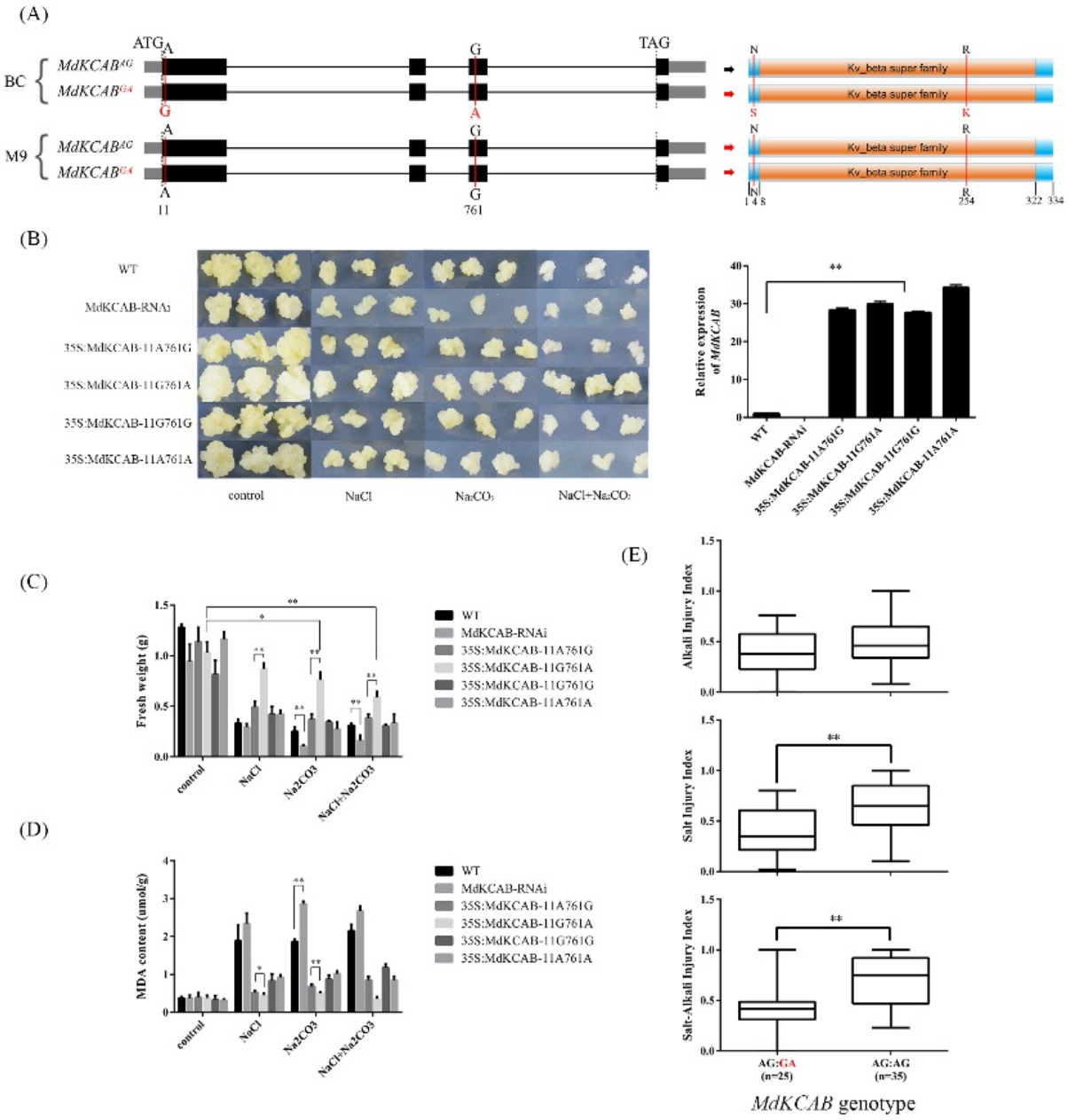

Figure 7

Schematic diagram showing genetic variations of the coding sequence of MdKCAB and the functional validation of these variations via callus transgenesis and genotype–phenotype association. A: Schematic diagram showing single nucleotide polymorphisms on the coding region and the changes in the encoded amino acids of MdKCAB. B: Images showing in vitro growth of transformed apple callus overexpressing variants of MdKCAB and the MdKCAB-RNAi line 14 days after treatment with NaCl, Na_2CO_3, or NaCl + Na_2CO_3. C and D: Column blots showing differences in the fresh weight of the apple callus (C) and malondialdehyde (MDA) content (D) 14 days after treatment with NaCl, Na_2CO_3 or NaCl + Na_2CO_3 among transformants overexpressing variants of MdKCAB or the MdKCAB-RNAi line. E: Box-plot showing injury indices of salt, alkali, and salt–alkali stress in F1 hybrids of ‘Baleng Crab’ × ‘M9’ with different genotype combinations of MdKCAB SNP11 and SNP761. Data are means ± standard deviation of three biological replicates. *, **, and *** indicate statistically significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

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