**Supplementary Figure legends**

**Figure S1. Quality and features of the RNA-seq datasets.** (**A**)Principal component analysis (PCA) of the RNA-seq samples. (**B**) Violin plots showing Log10-transformed FPKM measurements for all samples. Filled white circles represent the median, and the first and third quartiles are represented, respectively, by the lower and upper bold vertical lines. The lower and upper thin vertical lines respectively represent the lower and upper adjacent values. NN, optimal nitrate; NL, low nitrate; NH, high nitrate; R1–3, biological replicate number. (**C**)Normalized gene-body coverage of the RNA-seq libraries generated from soybean roots. For each library, average coverage at each transcript position is shown.

**Figure S2. Identification and comparison of nitrate-responsive DEGs in the soybean root.** (**A**) Up- and downregulated DEGs in each treatment group. (**B–C**) Venn diagrams showing the overlapping **(B)** upregulated and **(C)** downregulated DEGs among treatment groups.

**Figure S3. Categories and numbers of AS events are schematically illustrated by ASTALAVISTA program.**

**Figure S4. Venn diagrams showing the overlaps among differential alternative splicing (DAS) events for each AS type across treatment groups.** (**A**) A3SS, (**B**) A5SS, (**C**) RI, (**D**) SE, and (**E**) MXE. (**F**) Overlap between genes with AS events (DSGs and DEGs) under different nitrate treatments.

**Figure S5. Three representative nitrate-responsive AS events identified using NA-seq and validated using RT-PCR**.(**A–C**) Representative genes that switch between isoform 1 and isoform 2 in response to different nitrate concentrations: **(A)** malectin/receptor-like protein kinase (*Glyma.18G273100*); (**B**) unknown gene (*Glyma.19G147600*); and (**C**) splicing factor (*Glyma.06G324000*). (**D**) RT-PCR results and gene structures, visualized using the IGV browser (<https://software.broadinstitute.org/software/igv/>; Robinson et al., 2017).