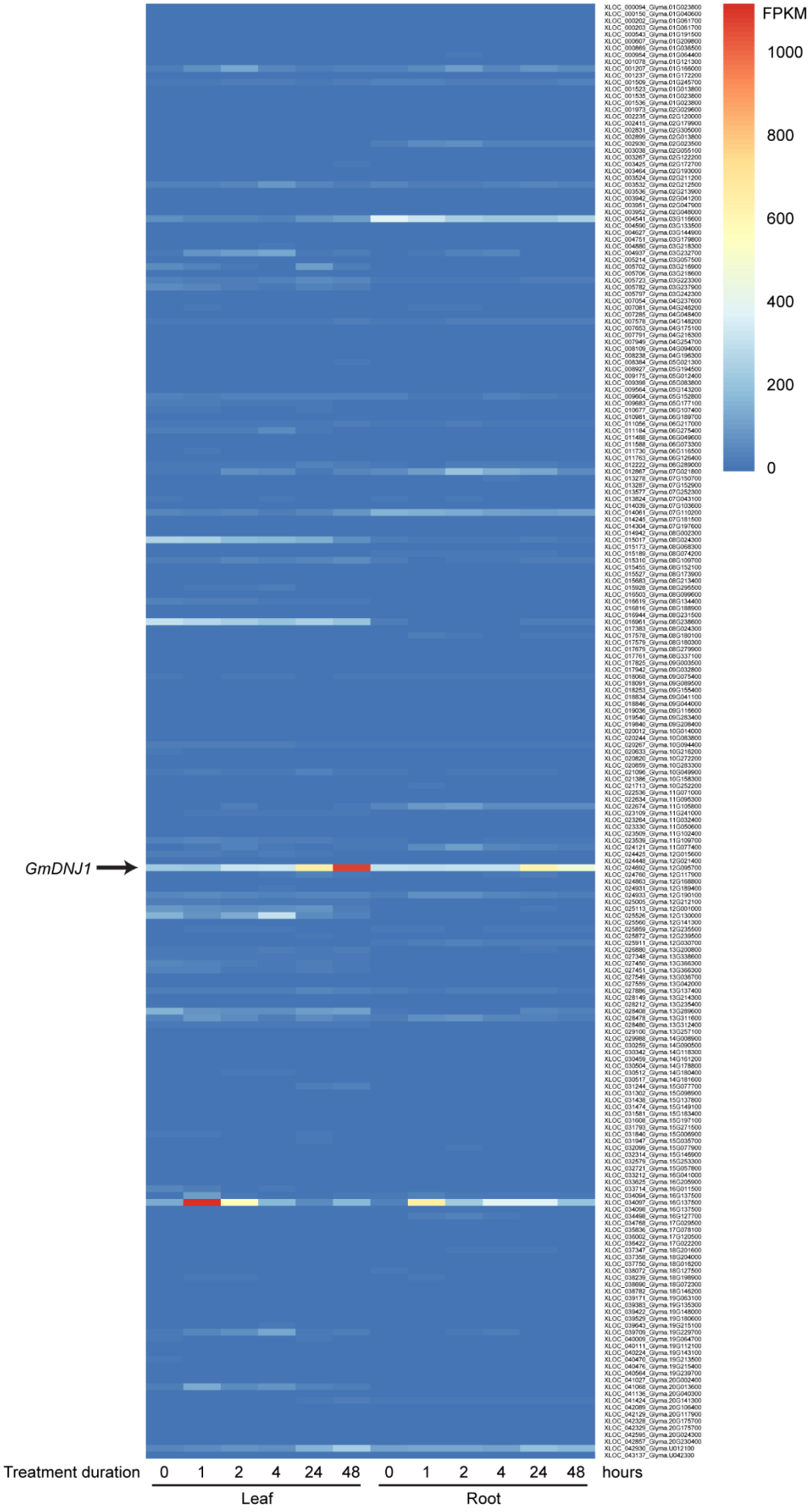
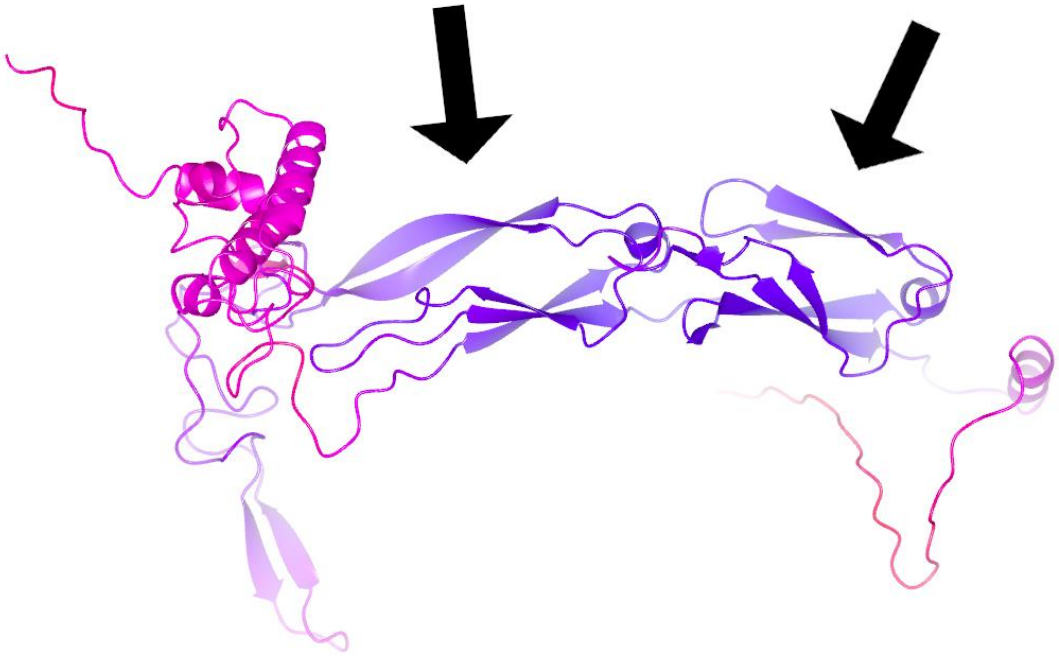
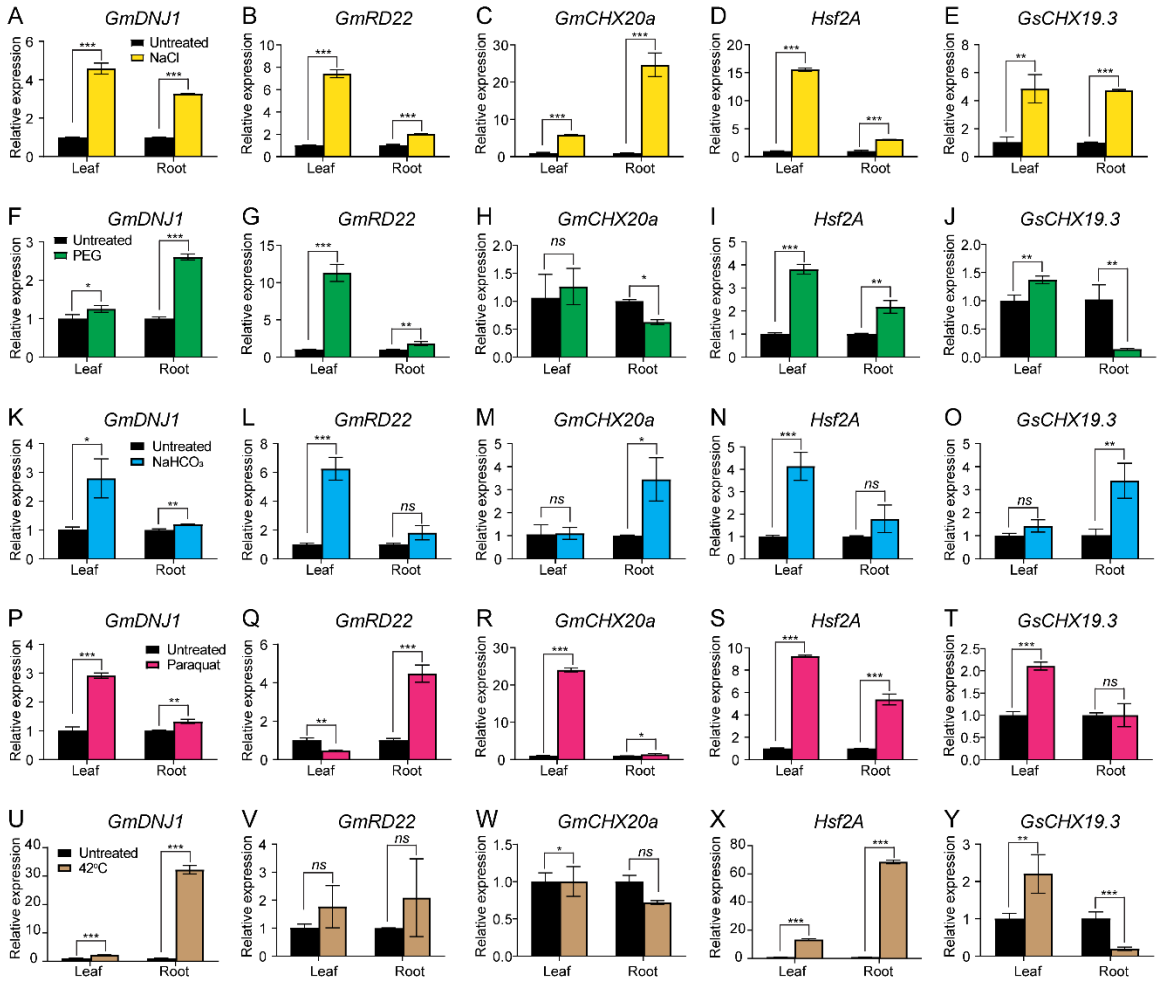

FPKM
1000
800
600
400
200
0
GmDNJ1
Treatment duration
0 1 2 4 24 48 0 1 2 4 24 48 hours
Leaf
Root

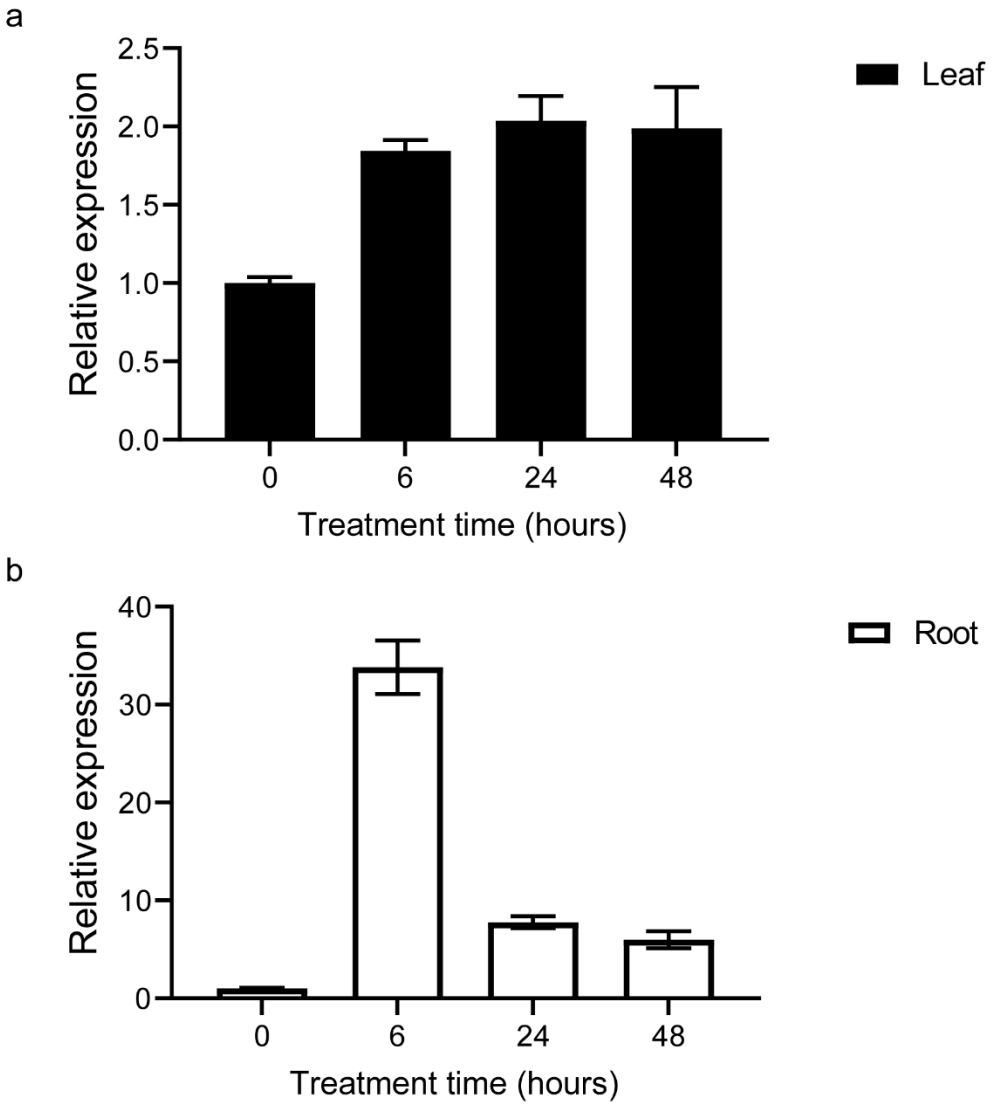
**Supplemental Figure S1. Expressions of soybean J-domain proteins in leaf and root under salt stress.** Expression data were extracted from a previous study on the soybean cultivar C08 (Liu et al., 2019). The expressions of genes annotated with PF00226 (DnaJ domain) were retrieved. In total, there were 224 DnaJ domain-containing genes found in the Williams 82 genome. FPKM values of 213 expressed genes corresponding to 205 J domain-containing genes were extracted from the Williams 82 database. The heatmap was generated with the pheatmap package of R.



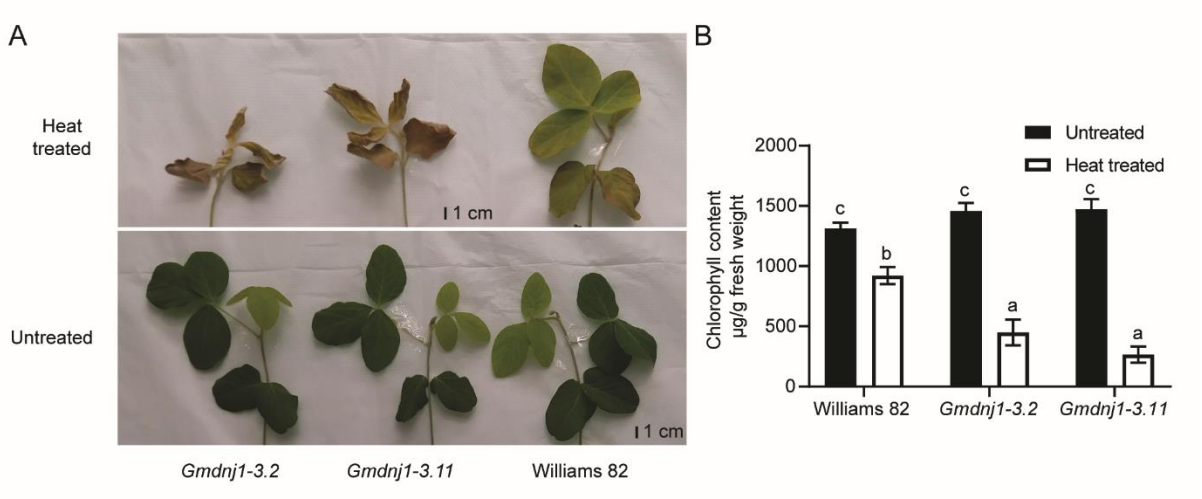
**Supplemental Figure S2. Predicted 3D structure of GmDNJ1.** The 3D structure was predicted using the web tool IntFOLD 5.0 (McGuffin et al., 2018;McGuffin et al., 2019), using a colour gradient from blue to red to represent the prediction accuracy from high to low. The predicted structure was presented and coloured with the software CCP4mg (McNicholas et al., 2011). The arrows indicate the two β-barrel structures.



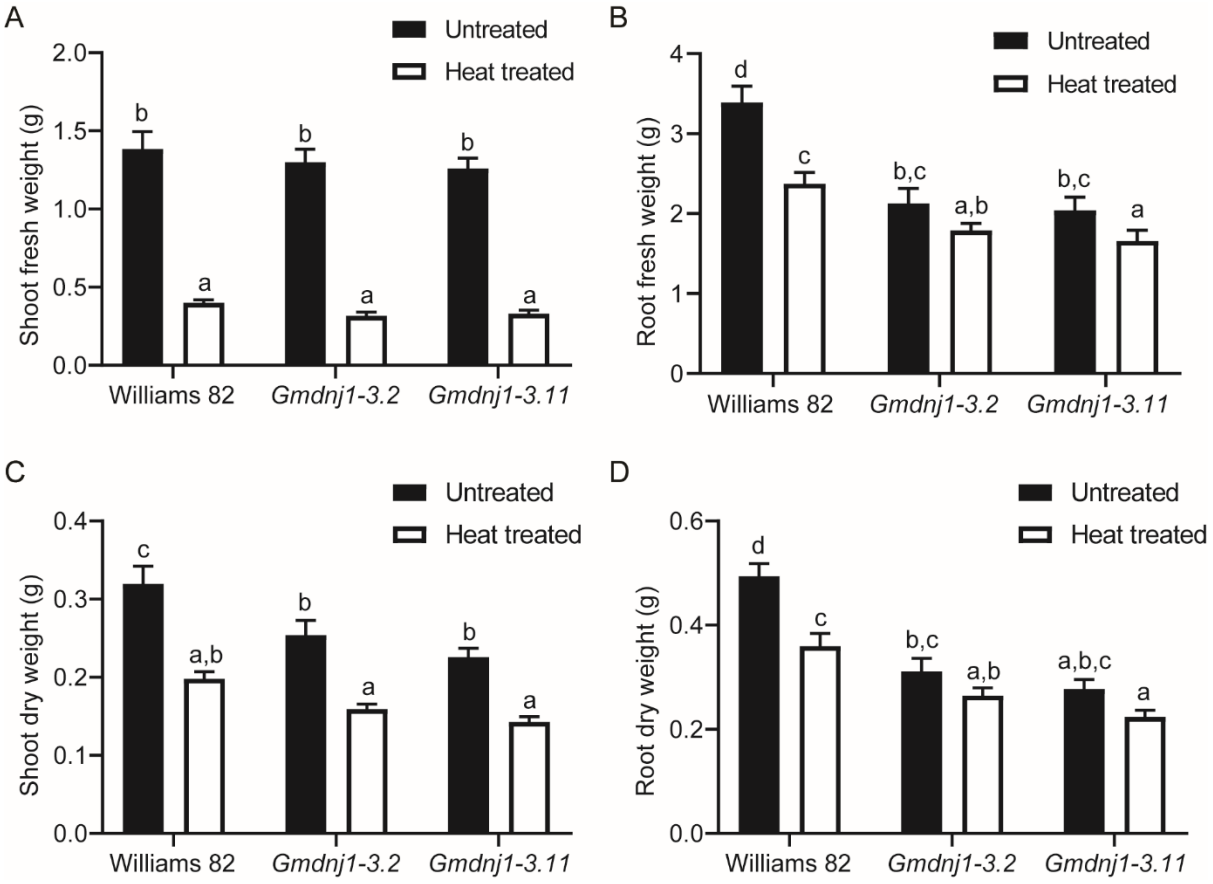

**Supplemental Figure S3. Biological replicate of the expression study of *GmDNJ1* under abiotic stress treatments.** First-trifoliate seedlings of *G. max* cultivar C01 were treated with (A-E) 9% NaCl for 4 h, (F-J) 5% PEG for 24 hr to induce osmotic stress, (K-O) 50 mM $NaHCO_3$ at pH 8.5 for 24 h, (P-T) 10 mM paraquat for 4 h to induce oxidative stress, and (D) heat stress at 42 $^{o}$C for 4 h. Expressions of *GmDNJ1* and other stress responsive genes in leaves and roots were analyzed by RT-qPCR. The expressions of *GmDNJ1* in the treated tissues were normalized to those in the respective untreated tissues. *α-tubulin* was used as the housekeeping gene for normalizing RNA input. Relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method. The error bar represents the standard deviation of three technical repeats. Two-tailed student's t-test was adopted to compare the expressions between untreated and treated samples. *, **, and *** indicates a significant difference at $p<0.05$, $p<0.01$, and $p<0.001$, respectively. *ns* means that there was no statistically significant difference.



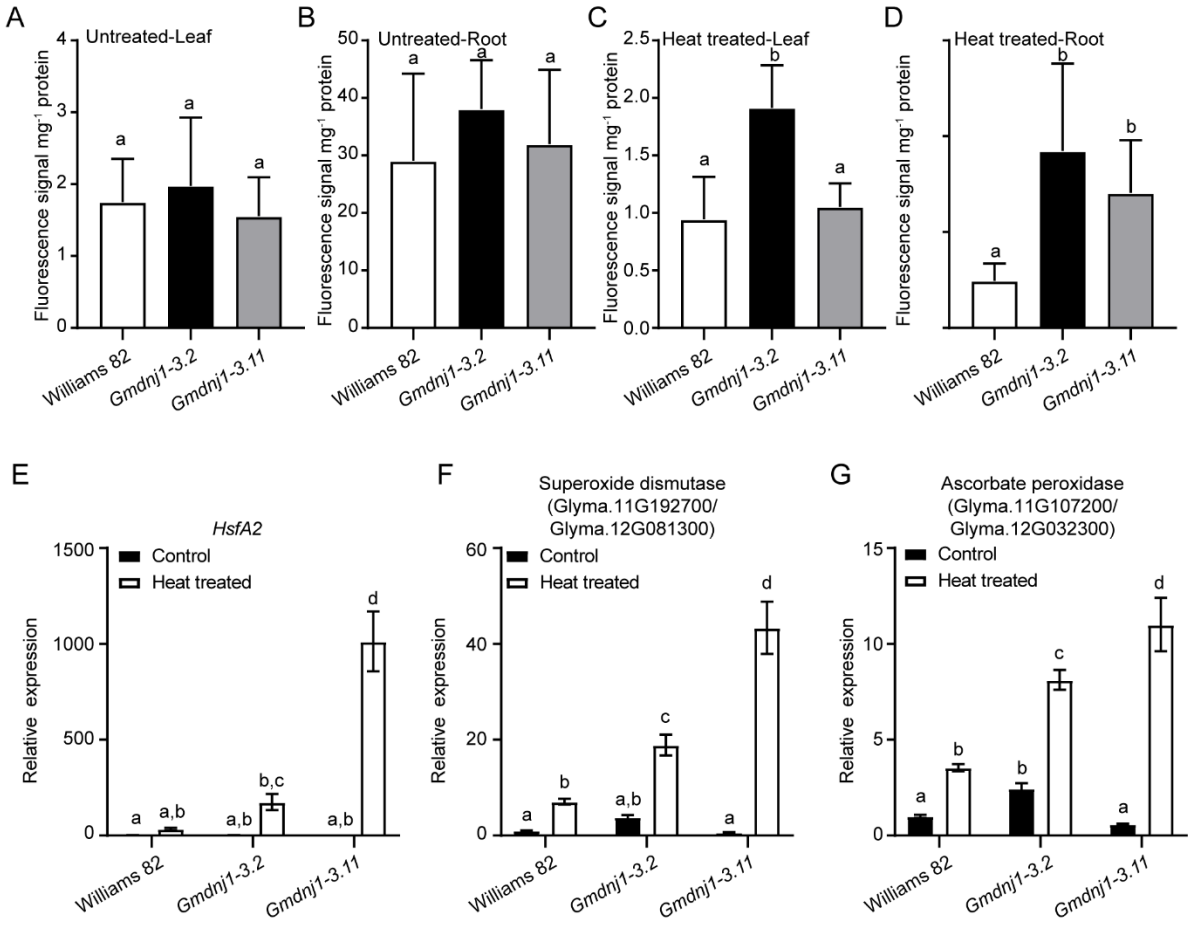

**Supplemental Figure S4. Expressions of *GmDNJ1* under heat treatment in Williams 82.** Two-week-old seedlings of *G. max* cultivar Williams 82 were heat-treated at 45 ºC for various durations. (a) Expressions of *GmDNJ1* in the leaf upon heat treatment. (b) Expressions of *GmDNJ1* in the root upon heat treatment. *Elf1b* was used as the housekeeping gene (Ma et al., 2013) for normalizing RNA input. Relative gene expression was calculated by the $2^{-\Delta\Delta CT}$ method. The error bar represents the standard deviation of four technical repeats.




**Supplemental Figure S5. Biological replicate of growth performance and chlorophyll contents of *Gmdnj1* mutant lines.** (A) Photos showing two-week-old *Gmdnj1* mutant plants treated at 45 ºC/28 ºC (heat-treated) and 28 ºC/28 ºC (untreated) following the 16 h/8 h light-dark cycle for 4 days. (B) Chlorophyll contents of the mutant lines with or without heat treatment. Data were assessed with one-way ANOVA followed by Tukey's post hoc test. Different letters above the bars indicate means that were significantly different at $p<0.05$. $N \geq 4$. Errors bars: SEM.




**Supplemental Figure S6. Biological replicate experiment for fresh weights and dry weights of *Gmdnj1* mutant lines under normal and heat treatment conditions.** (A) Shoot fresh weights, (B) root fresh weights, (C) shoot dry weights, and (D) root dry weights of wild type Williams 82 and two *Gmdnj1* mutant lines after 4 days of heat treatment at 45 ºC/28 ºC under the 16 h/8 h light-dark cycle. Data were assessed with one-way ANOVA followed by Tukey's post hoc test. Different letters above the bars indicate means that were significantly different at $p<0.05$. $N \geq 4$. Errors bars: S.E.M.




**Supplemental Figure S7. Biological replicate experiment for ROS contents and expressions of genes encoding ROS-scavenging enzymes in *Gmdnj1* mutant lines.** The ROS contents in leaf (A) and root (B) of untreated plants, and in leaf (C) and root (D) of heat-treated plants were compared by measuring the H2DCFCA fluorescence per unit protein in the extract. Expressions of *HsfA2* (E), heat shock element containing superoxide dismutase-encoding genes (F), and heat shock element containing ascorbate peroxidase-encoding genes (G) in root of *Gmdnj1* mutant were monitored. The data in A-D were analyzed with one-way ANOVA followed by LSD test. Different letters above the bars indicate means that are significantly different at $p<0.05$. $N \geq 4$. Error bar: S.E.M. The data in (E-G) were analyzed with one-way ANOVA followed by Tukey's test. Different letters above the bars indicate means that are significantly different at $p<0.05$. $N \geq 3$. Error bar: standard deviation.

**Supplemental Table 1. Primer information**

| Primer Name | Primer description | Primer Sequence (5'->3') |
|---|---|---|
| Tub-F | *α-tubulin* RT-qPCR forward primer | GAAGGCCTACCATGAACAGC |
| Tub-R | *α-tubulin* RT-qPCR reverse primer | GTGACGAGGATCACACTTGG |
| DNJ1-F | *GmDNJ1* RT-qPCR forward primer | TAAGACATCTTGGCCCATCC |
| DNJ1-R | *GmDNJ1* RT-qPCR reverse primer | CACAACCTTCTCTCCCTTGC |
| HSFA2-F | Glyma.04G052000 HsfA2 RT-qPCR forward primer | ACAGTCACACGAGGGAGGAAGT |
| HSFA2-R | Glyma.04G052000 HsfA2 RT-qPCR reverse primer | TCCCTCGAGTTGTGCTGTTGT |
| GmRD22-F | *GmRD22* RT-qPCR forward primer | GCCACAAGCAGAACTACCCTTATG |
| GmRD22-R | *GmRD22* RT-qPCR reverse primer | CACTGCTACCGCTTTAACCCTAAC |
| GmCHX20a-F | *GmCHX20a* RT-qPCR forward primer (Jia et al., 2020) | CGTATGCATCATCTTCTTTGG |
| GmCHX20a-R | *GmCHX20a* RT-qPCR reverse primer | GGTCATCCTTTTCAACAAACC |
| GsCHX19.3-F | *GsCHX19.3* RT-qPCR forward primer (Jia et al., 2017) | ACCCCTCAGACAACCCCG |
| GsCHX19.3-R | *GsCHX19.3* RT-qPCR reverse primer | TACGACGAATCGCACGCAT |
| SOD-F | Glyma.11G192700, Glyma.12G081300 Superoxide dismutase RT-qPCR forward primer | CGTCGCCACTCTCATCCAAGAA |
| SOD-R | Glyma.11G192700, Glyma.12G081300 Superoxide dismutase RT-qPCR reverse primer | AAACCATGAAGCCCCGGAGT |
| APX-F | Glyma.11G107200, Glyma.12G032300 L-Ascorbate peroxidase RT-qPCR forward primer | GGCGGCTTCCTGATGCTAAA |
| APX-R | Glyma.11G107200, Glyma.12G032300 L-Ascorbate peroxidase RT-qPCR reverse primer | TCCGTCCAAGGGCCATCAAA |
| Elf1b-F | GmELF1B forward primer | CCACTGCTGAAGAAGATGATGATG |
| Elf1b-R | GmELF1B reverse primer | AAGGACAGAAGACTTGCCACTC |

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