

Figure S1. (A) KEGG pathway enrichment analysis of DEGs and genes subjected to DAS events in Group 1 and Group 4. (B) Overlapping GO pathways between DEGs and genes subjected to DAS events in the cellular component category. Group 1 represents H₂O shoots vs. H₂O roots; Group 2 represents Pb roots vs. H₂O roots; Group 3 represents Pb shoots vs. H₂O shoots; and Group 4 represents Pb shoots vs. Pb roots.

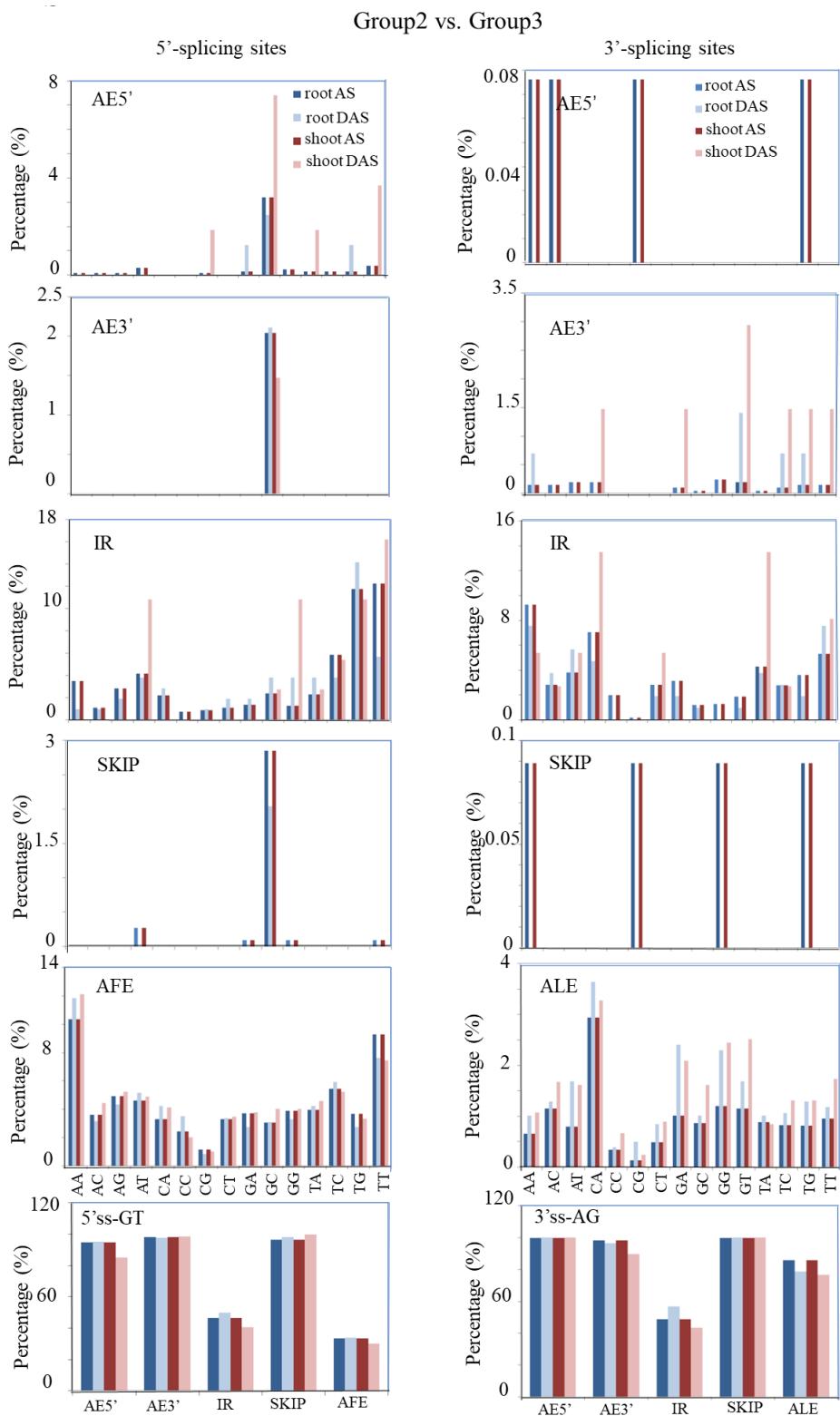


Figure S2. Frequency of traditional and nonconventional splice site selection in specific splicing events in Group 2 and Group 3 members between the shoots and roots. Group 1 represents H₂O shoots vs. H₂O roots; Group 2 represents Pb roots vs. H₂O roots; Group 3 represents Pb shoots vs. H₂O shoots; and Group 4 represents Pb shoots vs. Pb roots.

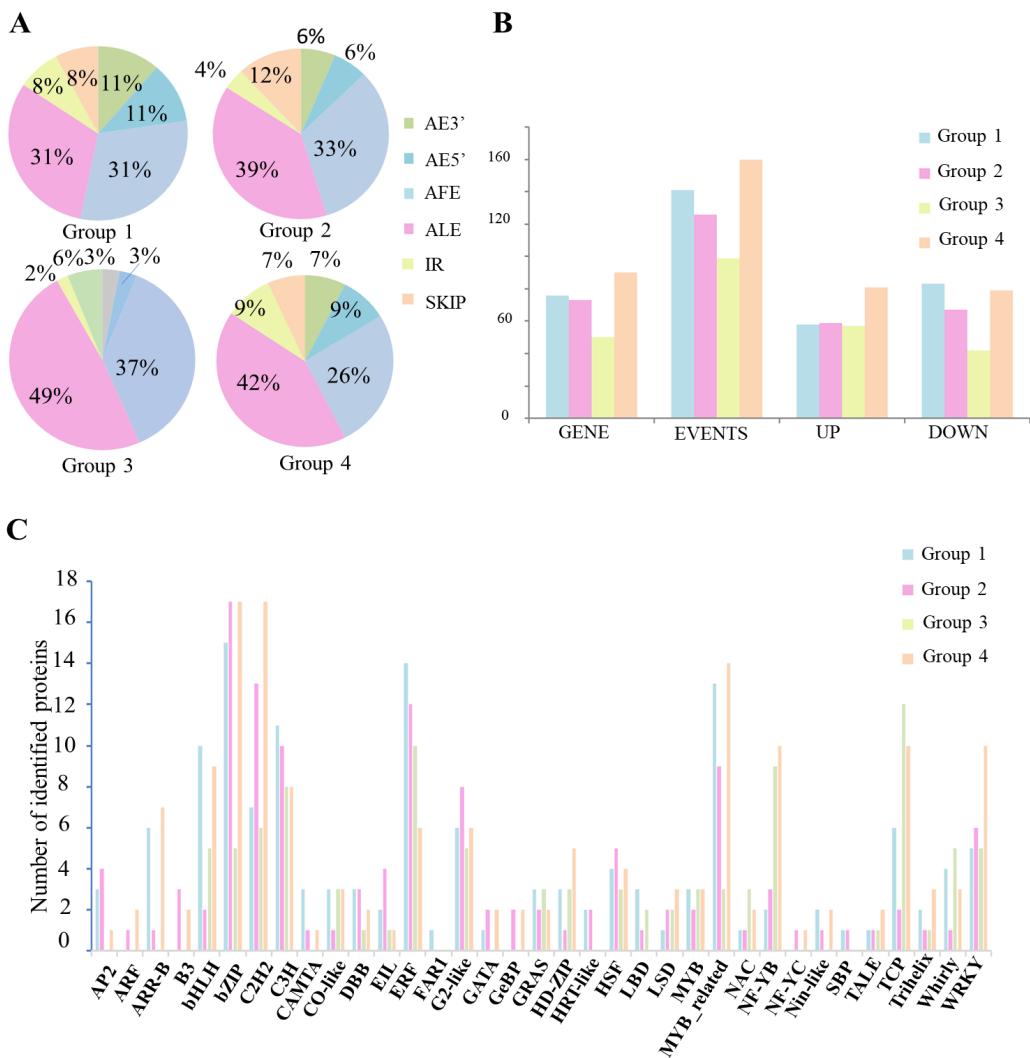
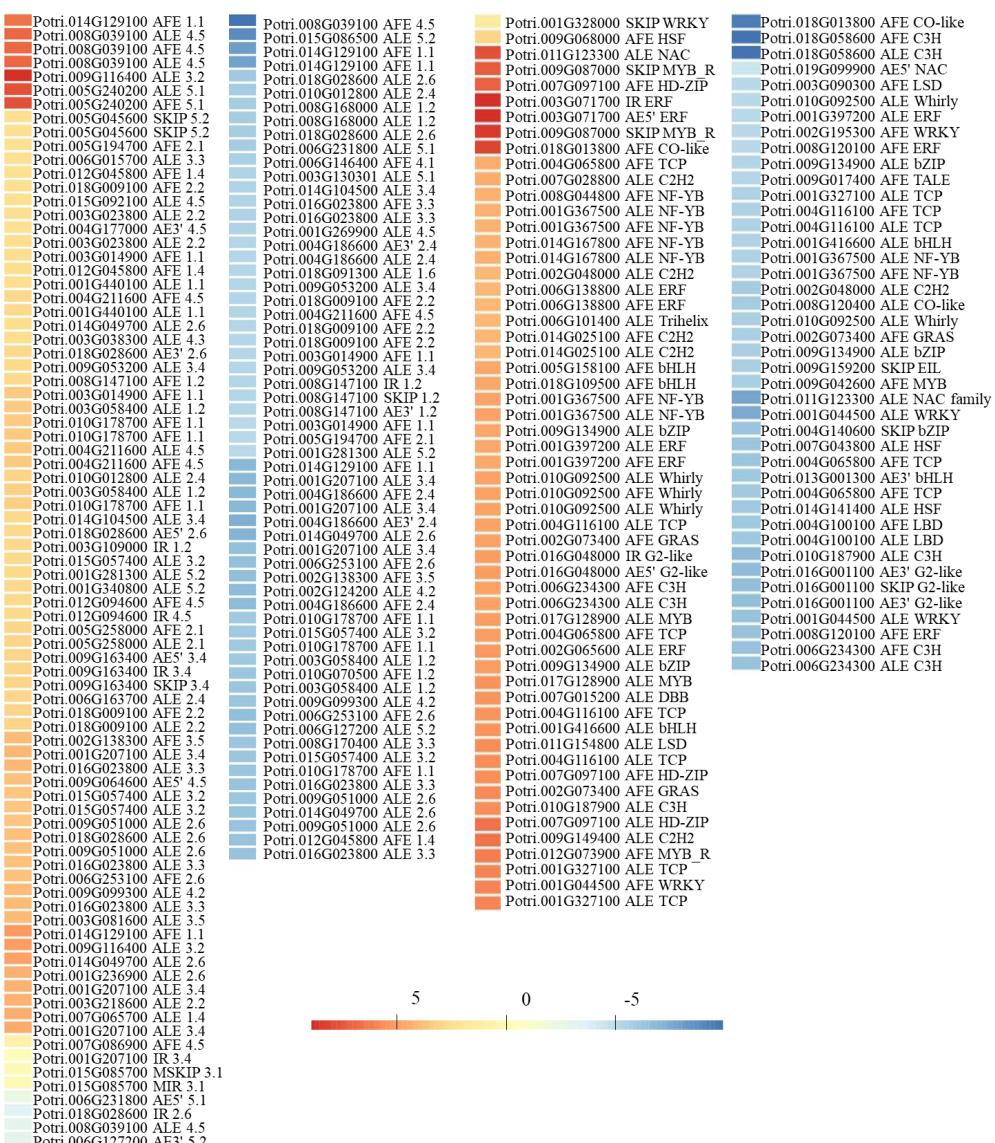
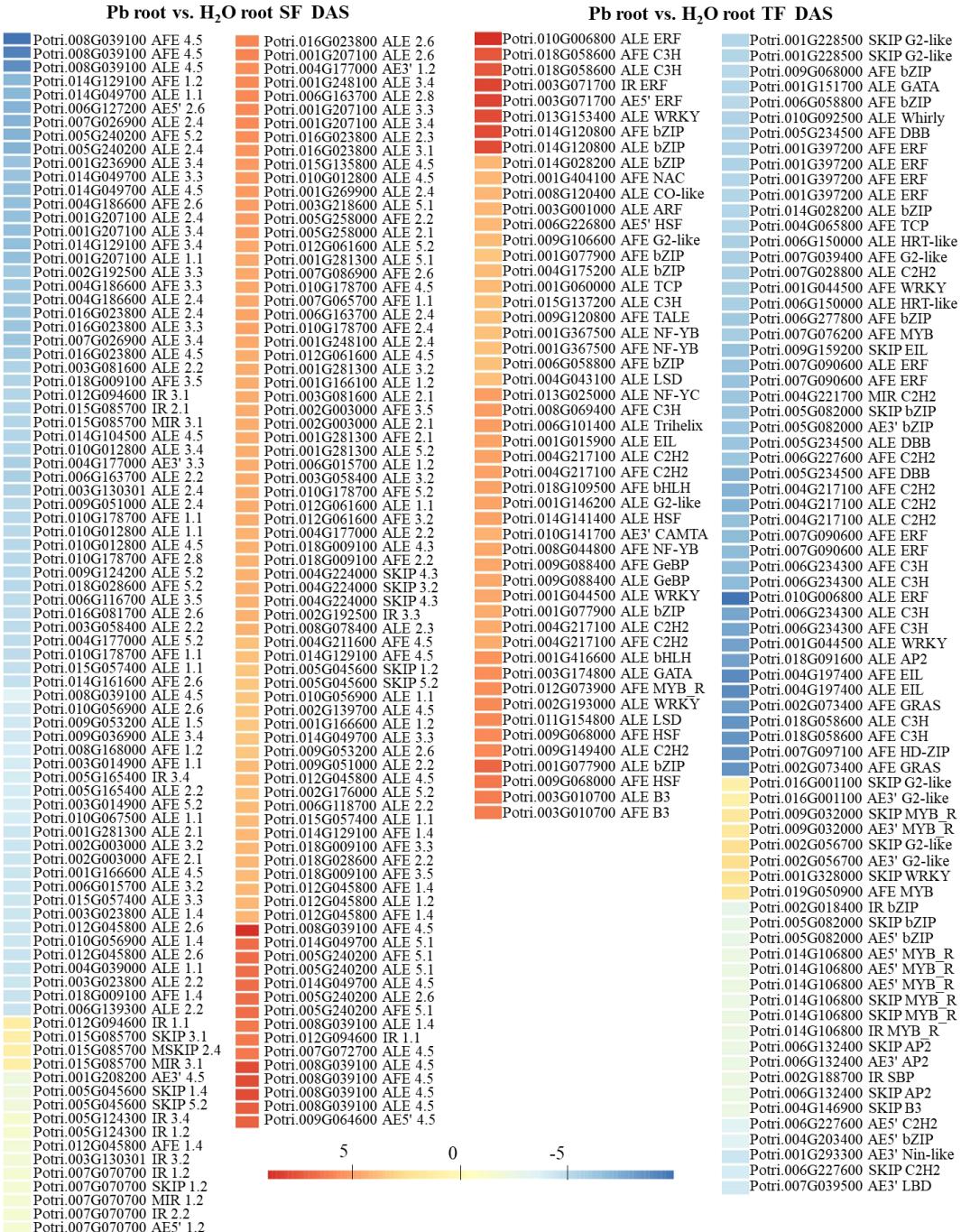


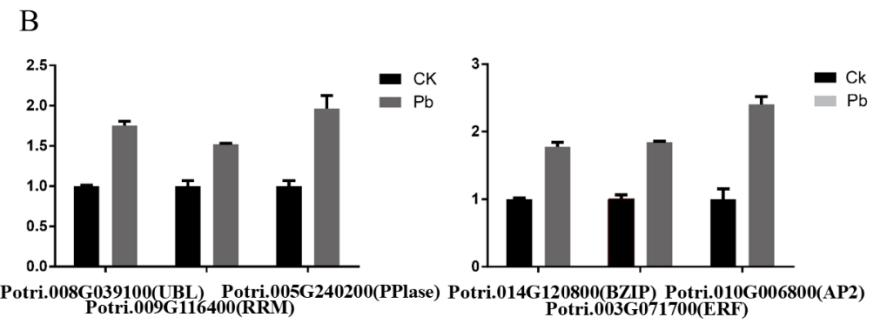
Figure S3. Splicing event statistics and groups of TFs. (A) Proportion of splicing events in each compared group. (B) Number of identified proteins in each TF family. (C) Numbers of genes, events, increased events and decreased events. Group 1 represents H₂O shoots vs. H₂O roots; Group 2 represents Pb roots vs. H₂O roots; Group 3 represents Pb shoots vs. H₂O shoots; and Group 4 represents Pb shoots vs. Pb roots.

A

Pb shoot vs. H₂O shoot SF DAS







C

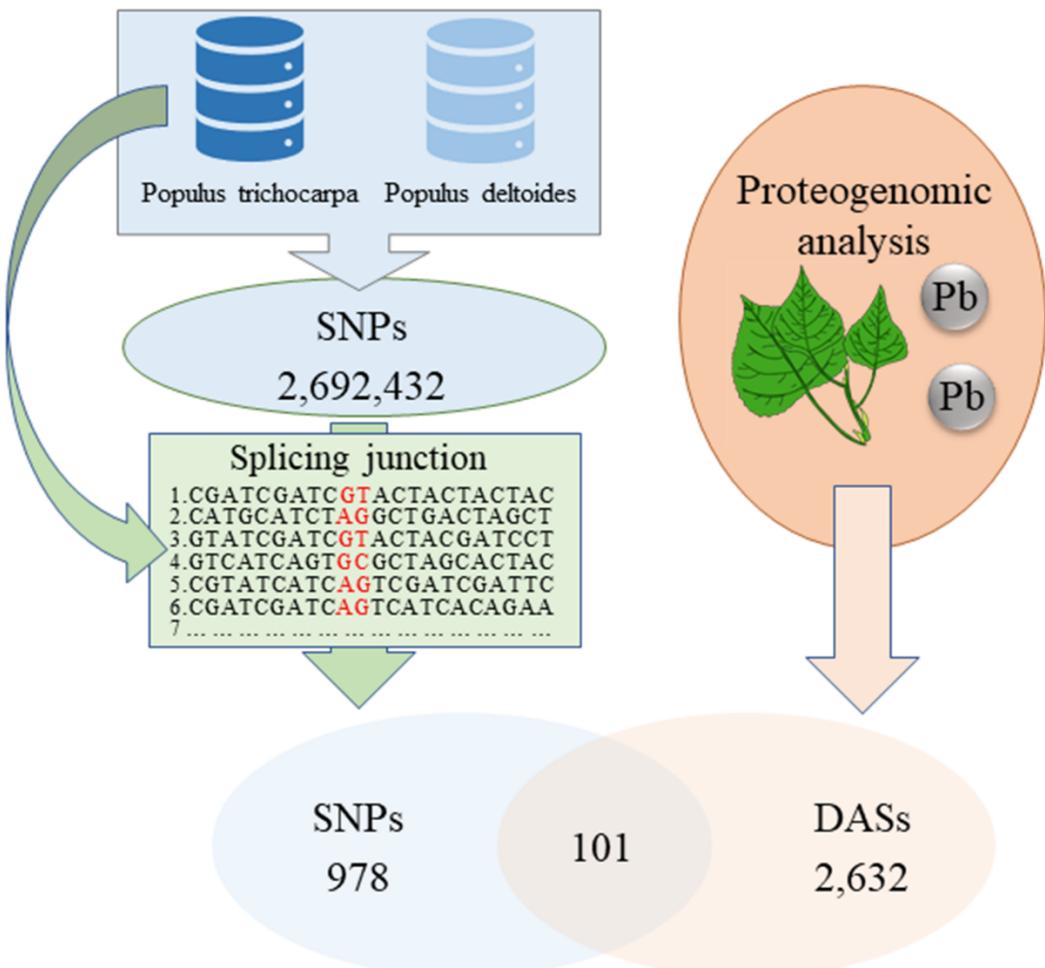
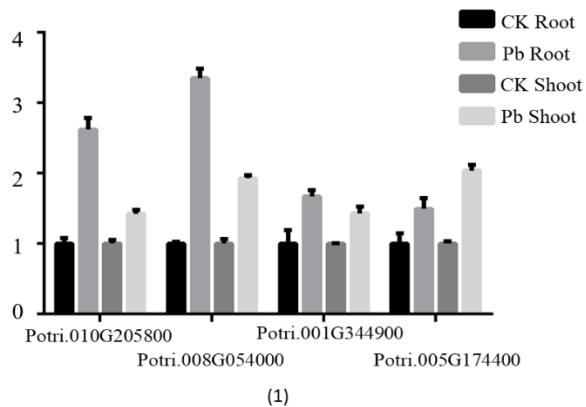


Figure S4. (A) Heatmap showing the expression levels of SF- and TF-encoding genes subjected to DAS events in Group 2 and Group 3. (B) RT-qPCR validation of the WRKY gene. (C) The process of SNP identification.

A**H2O Root VS. Pb Root****H2O Shoot VS. Pb Shoot**

B

(1)

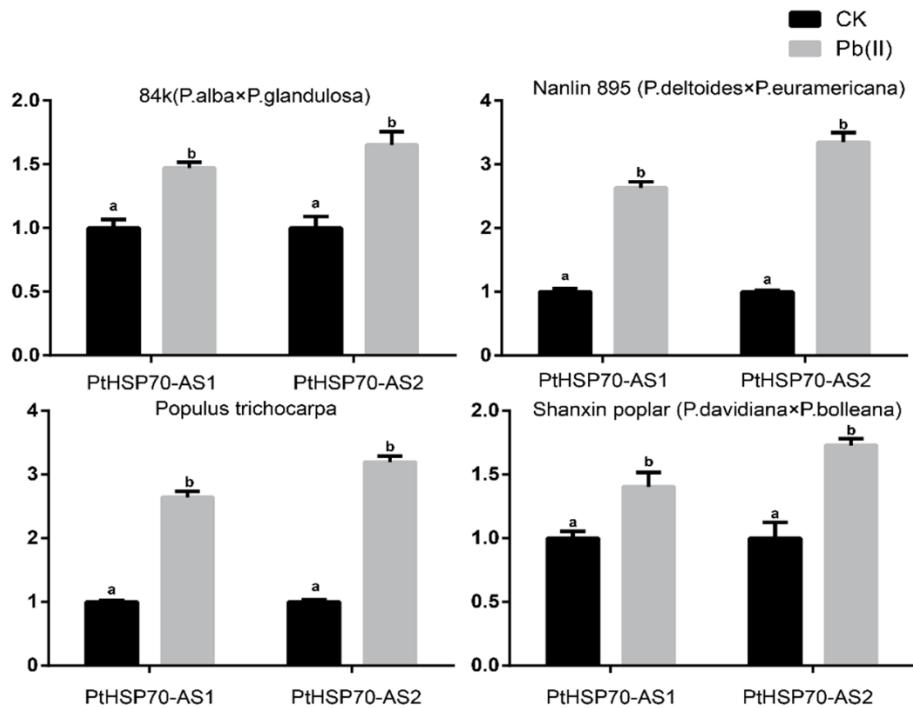
C

Figure S5. Correlation analysis of DEGs and DEPs. (A) Heatmaps of DEP and DEG correlations. (B) RT-qPCR validation of DEGs. (C) The expression of PtHSP70 under Pb resistance among 4 poplar species.

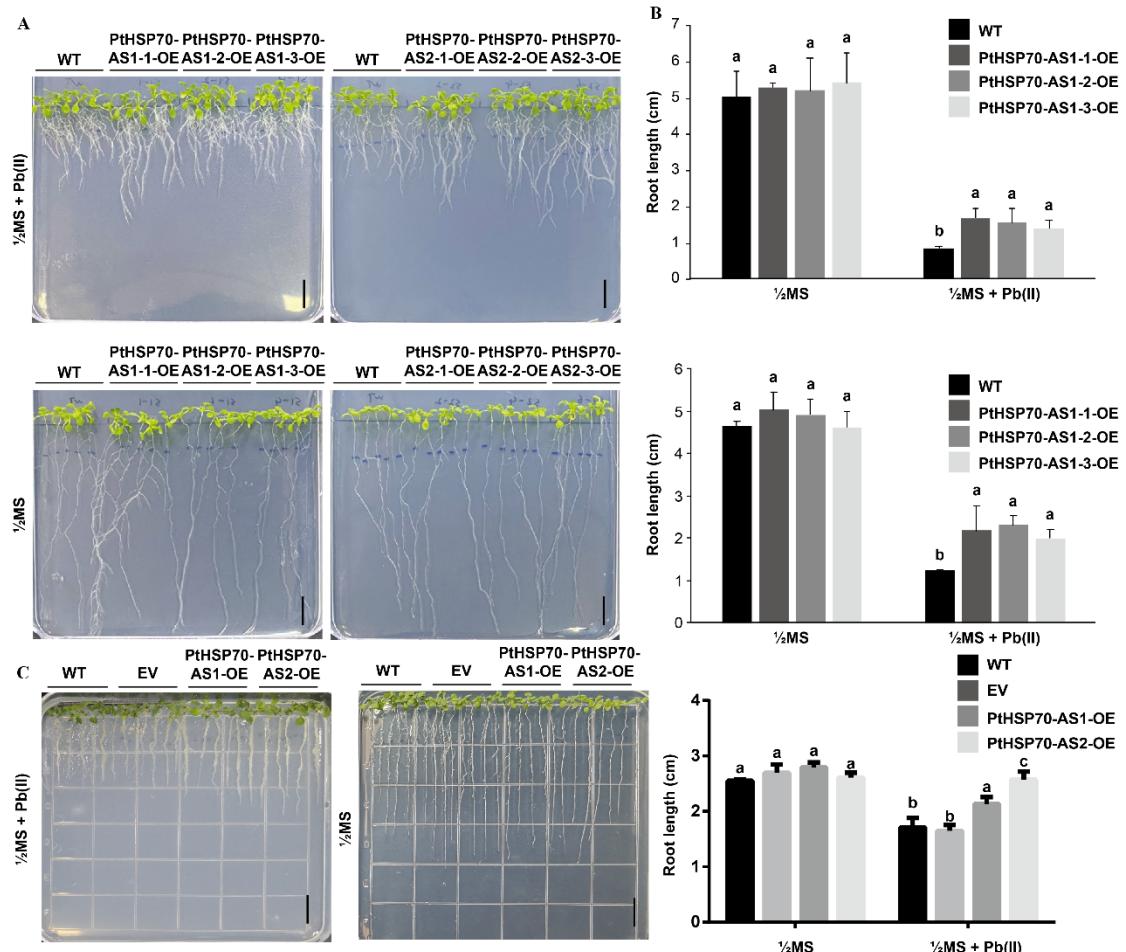


Figure S6. Phenotypic analysis of transgenic Arabidopsis overexpressing PtHSP70-AS1 and PtHSP70-AS2. (A, B and C) Phenotypic analysis of transgenic Arabidopsis overexpressing PtHSP70-AS1 and PtHSP70-AS2 and EV with and without Pb treatment. (A) Phenotype of transgenic Arabidopsis overexpressing PtHSP70-AS1 and PtHSP70-AS2. Scale bars denote 1 cm (B) Root length of transgenic Arabidopsis overexpressing PtHSP70-AS1 and PtHSP70-AS2. Values represent mean \pm SD ($n = 10$). Asterisks represent significant differences WT and transgenic lines (ANOVA, $P < 0.05$). (C) Phenotype of transgenic Arabidopsis of EV and the root length of them.

CK, control sample grown the absence of Pb; Pb, sample grown in the presence of Pb; WT, wild type; PtHSP70-AS1-1-3, transgenic Arabidopsis overexpressing PtHSP70-AS1 line 1-3; PtHSP70-AS2-1-3, transgenic Arabidopsis overexpressing PtHSP70-AS2 line 1-3.

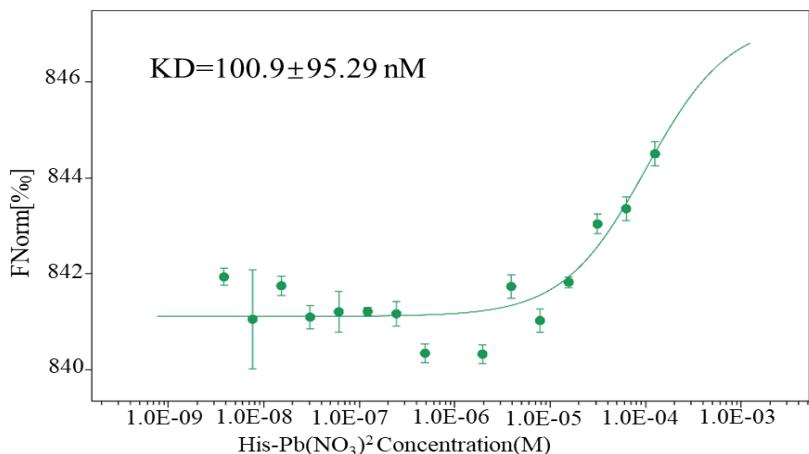
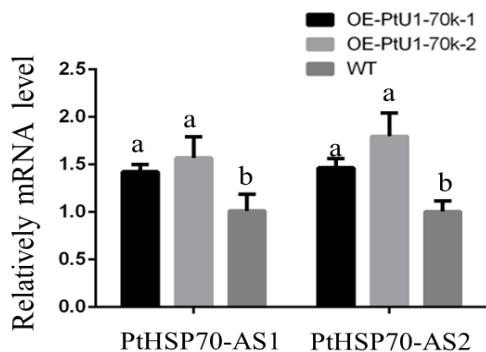
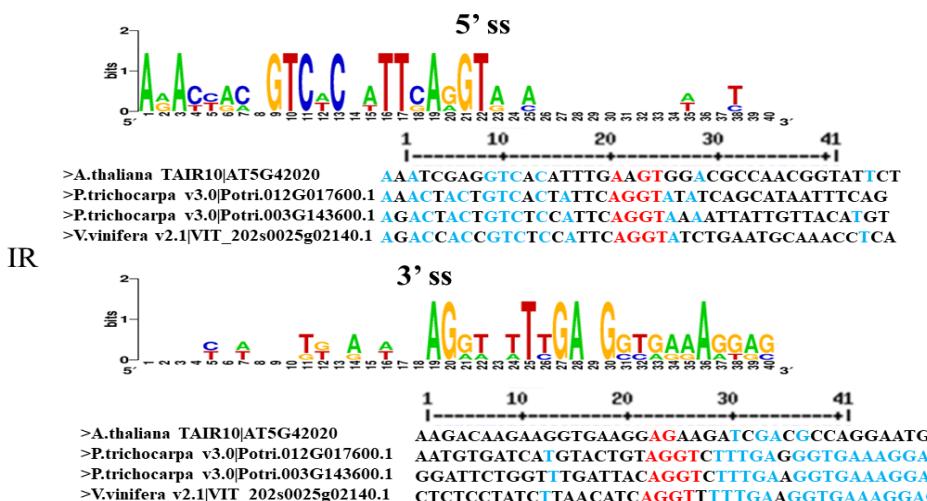
A**B****C**

Figure S7. (A) Results of the MST binding assay used to determine the Kd value for the binding of His proteins to Pb(II). (B) Results of the RT-qPCR validation of the expression of PtHSP70.1/PtHSP70.2 in transgenic poplar lines overexpressing PtU1-70k. (C) Analysis of conserved AS site sequences (IR events) in plant HSP70 genes. The flanking sequences (20 bp on each side, as indicated by coloured arrows in Figure 7(A)) of plant HSP70 genes were analysed to determine their consensus sequences

using WebLogo and multiple alignment.

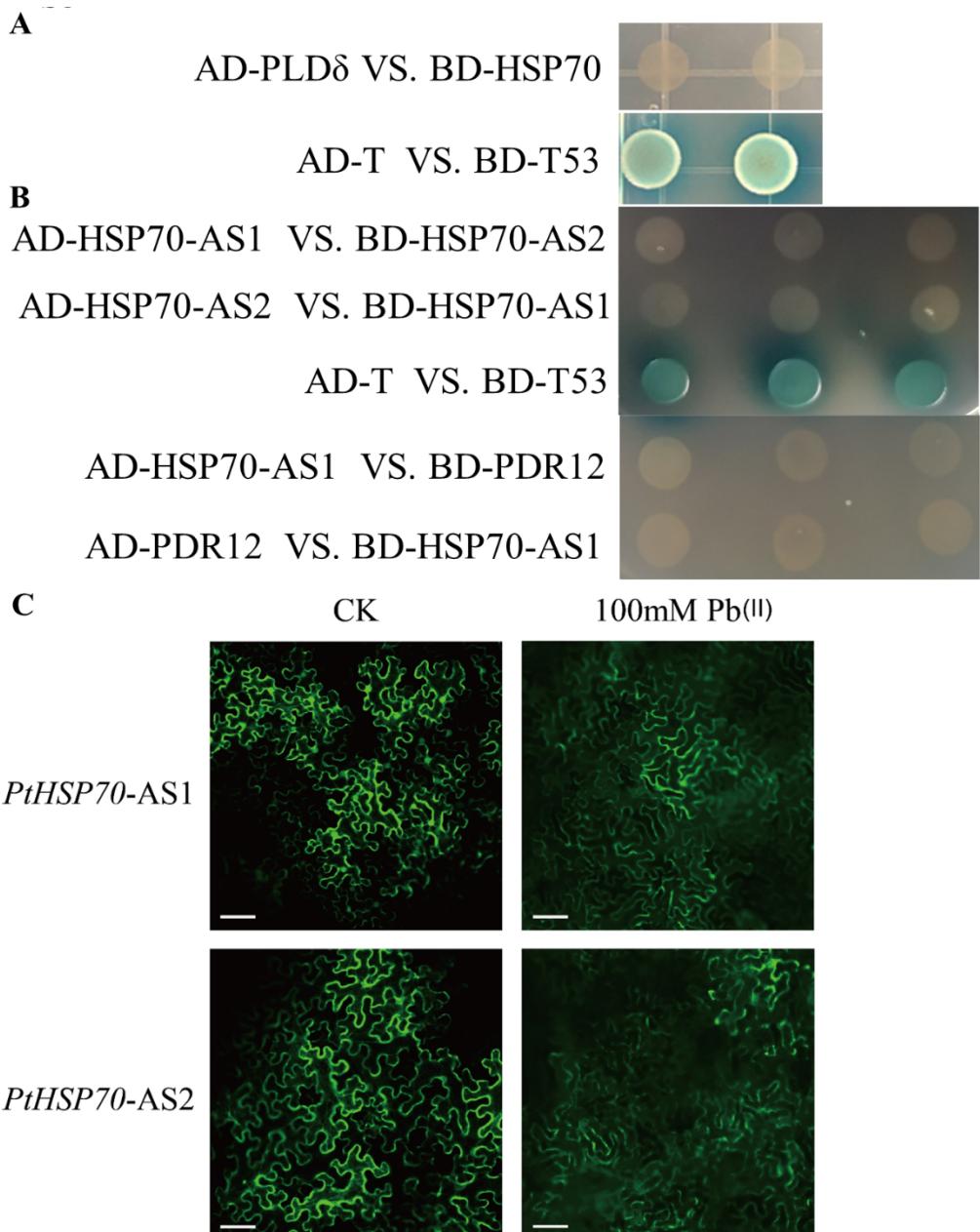


Figure S8. Analysis of the interactions via a Y2H assay and subcellular localization of PtHSP70. (A) The interaction between PtHSP70 and PtPLD δ (B) The interaction between HSP70-AS1 and HSP70-AS2 and HSP70 with PDR12. Plasmids were cotransfected into yeast cells and selected on SD-Leu/-Trp and SD-Leu/-Trp/-His/-Ade media. T and T53 were used as positive controls. (C) The subcellular localization of PtHSP70 with and without Pb(II).

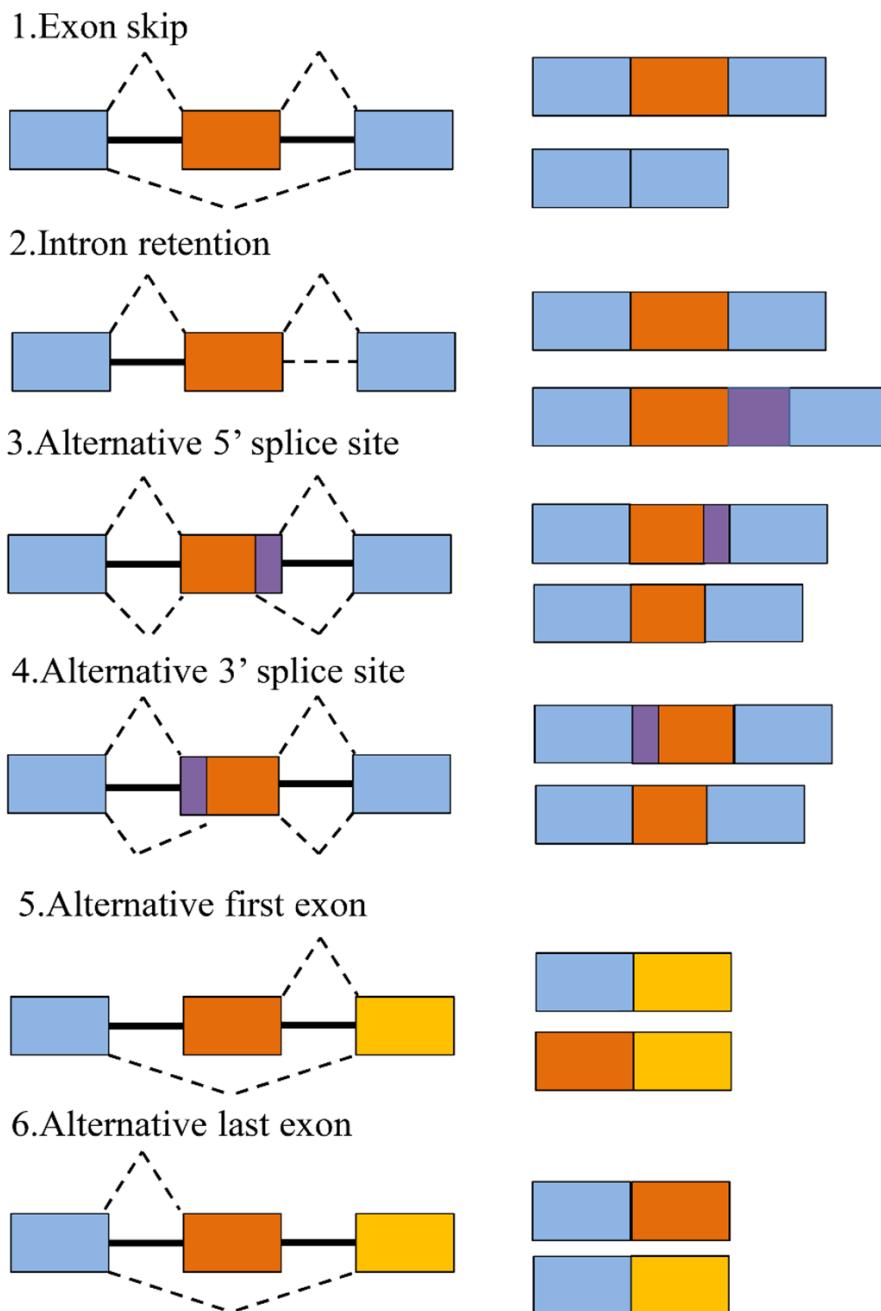


Figure S9. Pattern diagram for identifying AS events.



Peptide	Protein information
FTDASVQSDIK	PtHSP70.1
RFTDASVQSDIK	PtHSP70.1
VISGPGDKPMIGVTYK	PtHSP70.1
EIAEAYLGTALK	PtHSP70.2
KIEDAIDQAIQWLDSNQLAEADDEFEDK	PtHSP70.2
NALENYAYNMR	PtHSP70.2

Figure S10. The spectrum information of SWATH-MS.

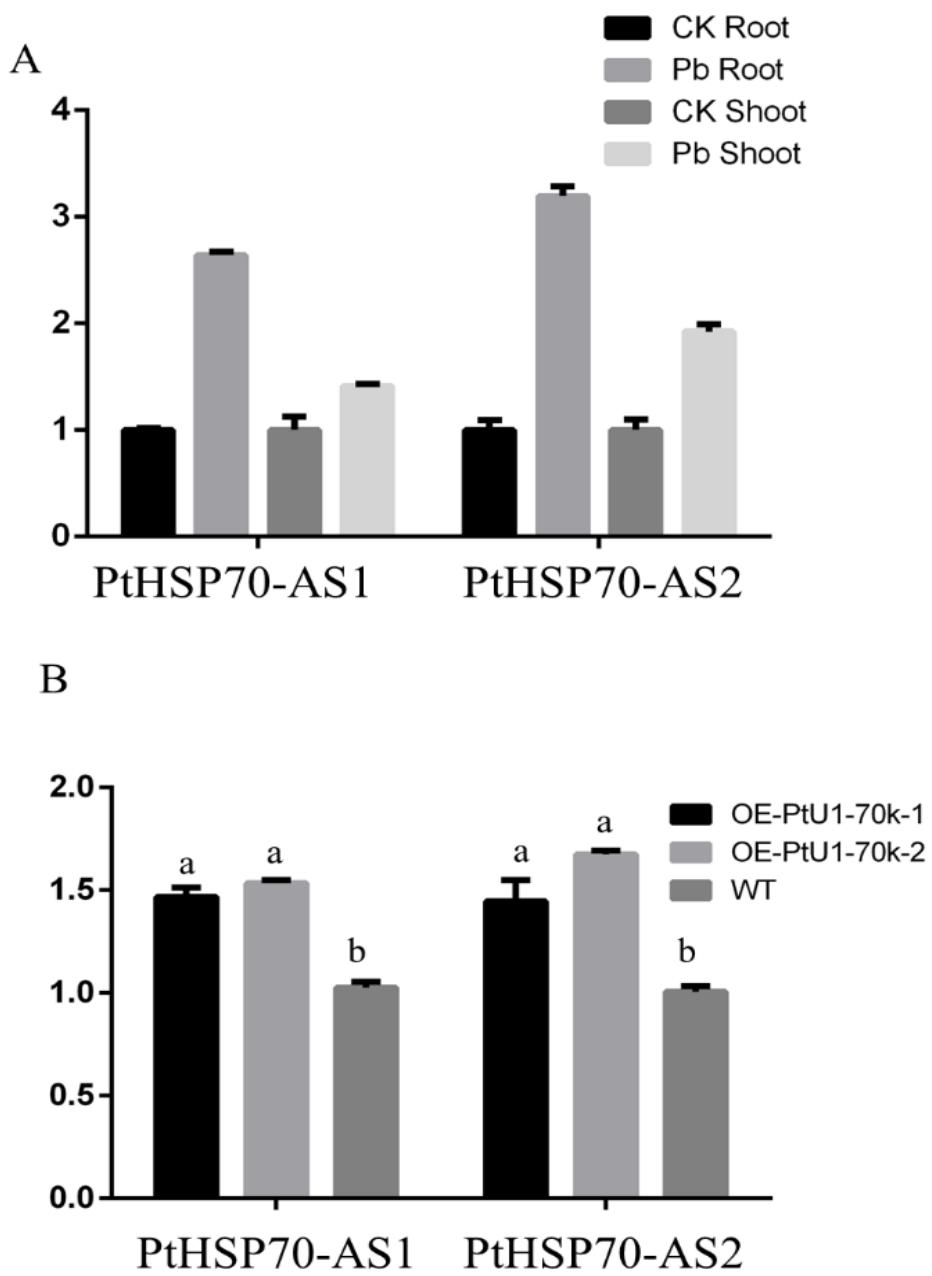


Figure S11. The expression of RT-qPCR in the reference of *PtACT6*.