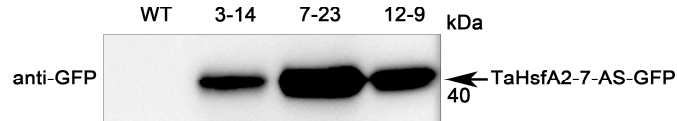
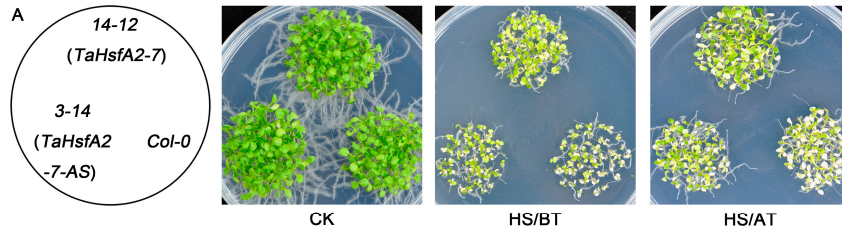


**Figure S1.** The effect of C-terminal hydrophobic sequence on the transcriptional activation of TaHsfA2-7-AS. Transcriptional activation analysis of D-TaHsfA2-7-AS in yeast. Fusion proteins of the GAL4 DNA-binding domain (BD) and D-TaHsfA2-7-AS were expressed in the yeast strain AH109. The transformed yeast cells were spotted onto SD/-Trp and SD/Trp-/His-/Ade-media. The plates were incubated at 30°C for 3 days. The pGBKT7 and pGBKT7-P53 vectors were used as negative and positive controls, respectively.



**Figure S2.** Protein detection of TaHsfA2-7-AS-GFP in transgenic Arabidopsis. The accumulation of TaHsfA2-7-AS-GFP in transgenic lines was detected with anti-GFP antibody (1: 5000, Sigma). Nontransgenic plant was used as control.



**Figure S3.** Thermotolerance assays of two splice variants in Arabidopsis. The phenotypes of WT and transgenic seedlings after basal thermotolerance (BT, 45 °C for 1 h, placed at 22 °C for 8 days) and acquired thermotolerance (AT, 37 °C for 1 h, incubated at 22 °C for 2 days, 46 °C for 1 h, placed at 22 °C for 8 days) treatment. The plants were photographed 8 d after different HS.

**Table S1.** Primers used in this study.

Assays	Gene	Forward primer (5'→3')	Reverse primer (5'→3')
RT-PCR	TaHsfA2-7-AS	ATGGACCGGGTGCTGCTG	CTACGCGTCGAAACATCT
RT-qPCR	TaHsfA2-7-AS	CTAGATGGATCAAGACACCGGCTGA	CATCTTCAGAAGCTGTCTCTGGC
	TaRP15	GCACACGTGCTTTGCAGATAAG	GCCCTCAAGCTCAACCATAACT
Transactivation analysis	TaHsfA2-7-AS	TCAGAGGAGGACCTGCATATGATGGACCGGGTGCT G	TCGACGGATCCCCGGGAATTCCTAGAACAAATAAG C
Subcellular localization	TaHsfA2-7-AS	GAGAACACGGGGGACTCTAGAATGGACCGGGTGCT G	GCCCTTGCTCACCATGGATCCGAACAAATAAGCAG G
Genetic transformation	TaHsfA2-7-AS	GAGAACACGGGGGACTCTAGAATGGACCGGGTGCT G	GCCCTTGCTCACCATGGATCCGAACAAATAAGCAG G
Protein purification	TaHsfA1	CGTGGATCCCCGGAATTCATGGACGGCGGGTTGC TGCGGTGGC	ATGCGGCCGCTCGAGTCGACTCACCTCTATGGTTG GATGACAGCA
Thermotolerance assays in yeast	TaHsfA2-7-AS	GGGAATATTAAGCTTGGTACCATGGACCGGGTGCT G	TACATGATGCGGCCCTCTAGACTAGAACAAATAAG C
Labeled probe	HSE1	GCGCCGCGCGGCCCAAGAACTTCCGCCCGGCCCG CGTCC	GGACGGGGGCCGGGGCGGAAGGTCTCTGGGCCGCGC GGCGC
	HSE2	CGGCCCCGGCGTCCAGAAAGCTTCCGAGCCGGCCGG TCACG	GGACGGGGGCCGGGGCGGAAGGTCTCTGGGCCGCGC GGCGC
	HSE3	AAGTGATATTTGAGAGAAAATTCCTTATTTGACACT ATCTTAA	TTAAGATAGTGTCAAATAAGGAATTTTCTCTCAAAT ATCACTT
Labelled mutant probe	HSE1	GCGCCGCGCGGCCCAAAAAATTACGCCCGGCCCG CGTCC	GGACGGGGGCCGGGGCGTAATTTTTTGGGCCGCGC GGCGC
	HSE2	CGGCCCCGGCGTCCAAAAAATTACGAGCCGGCCGG TCACG	CGTGACCGGCCGCTCGTAATTTTTTGGACGCCGGG GCCG
	HSE3	AAGTGATATTTGAGAGAAAATTACTTATTTGACACT ATCTTAA	TTAAGATAGTGTCAAATAAGTAATTTTTTCTCAAAT ATCACTT